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The Bark Beetle Complex Associated with Lodgepole Pine Slash in Alberta[1]

Part IV—Distribution, Population Densities, and Effects of Several Environmental Factors

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Introduction

The investigation reported in this series was carried out with the object of determining the bark-beetle complex associated with slash accumulated under different cutting methods and the biologies, distribution, and abundance of the more important bark-beetle parasites and predators throughout the experimental area. This area is in the region termed by Halliday (1937), the B19 or foot-hills section of the Boreal Forest. The experimental area contained a pure stand of even-aged (80 years) lodgepole pine, *Pinus contorta* Dougl., var. *latifolia* Engelm., and is located near Strachan, approximately 20 miles southwest of Rocky Mountain House, Alberta.

The experimental area was comparatively uniform throughout in respect to site conditions, stems per acre, and diameter classes prior to logging. By spring, 1952, however, the environment had been changed by various types of cutting carried out the preceding winter. This investigation was concerned with bark beetles as they occurred in five different cutting blocks i.e. Block I – which was thinned from below, Block II – thinned from above, Block IV – a diameter limit cut to 6.5 inches, Block VI – a seed tree cut which left a residual stand of 10 trees per acre, Block VIII – a shelterwood cut. Roughly there were three contrasting environments among the five blocks investigated. Block IV was well shaded but contained very little slash. Blocks I and II were shaded but contained large volumes of slash. Blocks VI and VIII were exposed and contained large volumes of slash. Blocks III and VII were not included because logging had not been completed at the time the study was initiated. Block V was the untouched control block.

Three earlier papers dealt with the biologies of the more important scolytids, their predators and parasites. This paper is concerned with the manner in which the bark beetles dispersed throughout the newly logged blocks, and the effect of several factors on this distribution. The most important species was *Ips pini* Say which was the most suitable for analytical study. Accordingly the data concerning this species form the basis for the conclusions regarding distribution in the slash. *Hylurgops rugipennis* (Mann.) was the only insect in the stumps numerous enough to allow an adequate estimate of the population in that habitat.

Materials and Methods

Sampling coverage and techniques:

Logging in the experimental area was conducted during the fall and winter of 1951-52. A cruise was carried out within the untouched stand adjacent to the experimental area in the spring of 1952, before seasonal activity of the bark beetles had commenced. These cruise data provided information on the total number of bark-beetle infested trees per acre before the effects of the logging

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operation became evident. Six infested trees were selected for stem analyses. These were in the 5-6 inch diameter class which roughly represented the mean d.b.h. of the stand. (Calculated mean d.b.h. of the stand was 5.85 inches.) After being felled, one foot sections at intervals of three feet along the stem were debarked. The number of living and dead scolytids, by species and stage of development was recorded. The total bark surface of an average tree was determined, utilizing measurement data from the six stem-analysis trees. Cruises were carried out again in the fall of 1953 and 1954.

Insect populations which became established in the fresh slash during the spring and early summer of 1952, surviving populations in the late summer and fall, and surviving populations in the spring of 1953, were determined from data collected during a series of slash samplings. Slash sampling was carried out in the five blocks. Within each block, ten circular plots 50 feet in diameter were established, and within each of these plots, ten pieces of slash and five stumps were tagged. The sampling unit was one linear foot of slash. The following information was recorded: diameter; bark characteristics; position of the slash sample, aerial or on ground, and direction of the main axis; number of strikes by species on each aspect; number of adults, larvae, and pupae on each aspect by species. On six of these plots, the total bark area of the slash and total number of stumps were measured. This last information was used to estimate total populations per acre.

Slash samples were taken again in the spring of 1953. Duff sampling was necessary at this time because some overwintering adults hibernated in the duff. It had been found earlier that mature *Ips pini* adults dropped directly from the slash to the duff beneath. The duff samples, each 10 inches square were collected from beneath five pieces of slash in each of the 10 plots in Block I. Each square foot of duff under the slash was considered to be analogous to one linear foot of slash.

Field techniques for study of subcortical temperatures

Two stations were established in August, 1952, for subcortical temperature readings; the first station in an exposed location, the second, 200 yards from the first was in a shaded location. Pieces of slash, three inches in diameter were arranged in four ways: the main axis lying in an east-west direction and lying in a north-south direction, both on the ground and 18 inches above the ground.

Leads were inserted into bark-beetle galleries on the four aspects of each piece of slash. Subcortical and air temperature readings were taken with a Rubicon potentiometer. The lead from the potentiometer was number 24 gauge copper-constantan thermocouple wire, the leads into the bark-beetle galleries were number 30 gauge. Readings were taken once a day commencing at 2:30 p.m. when subcortical temperatures recorded on aspects of slash lying in an east-west direction approached the maximum for the day. The temperatures recorded on the east aspect of slash lying in a north-south direction were lower than would have been recorded had the readings been taken earlier in the day.

Analysis and Results

Distribution of *Ips pini*

The nature of the bark-beetle distribution was determined from the variation in population density on a linear foot basis. To determine if each linear foot or unit of measurement was exposed to infestation in a random manner, a single diameter class was selected and the number of strikes on each linear foot of sample was tabulated. A single diameter class was selected because susceptibility to attack by bark beetles was found to vary with diameter of the slash.

TABLE I
Relation of Mean Number of Strikes (*Ips pini*) per Square Foot of Bark Surface to Diameter of Slash

Diameter Class (ins.)	Block I	Block II	Block IV	Block VI	Block VIII	Mean
2.0–2.4.....	2.0	0.4				1.2
2.5–2.9.....	3.7	0.8	0.2	2.4	0.9	1.6
3.0–3.4.....	4.1	1.0	0.7	1.2	3.2	2.0
3.5–3.9.....	3.6	1.7	0.9	1.9	1.8	2.0
4.0–4.4.....	4.5	2.7		2.9	2.2	3.1
4.5–4.9.....	10.50	5.2		3.3	4.1	5.8

A relationship between the diameter of the slash and frequency of attacks by *Ips pini* adults is indicated by Table I. In all blocks the frequency distribution of different diameter classes approximated a normal distribution in the statistical sense. This reduced the likelihood that the greater density of attack in the larger diameter class might have been due to a greater total surface area of bark or greater accessibility.

The 3.5 inch to 3.9 inch class was found to represent most consistently, both in number of pieces and average strikes, the frequency distribution within the area. Three empirical distributions were tested: Poisson, negative binomial, and Neyman's contagious. Only Neyman's fitted the actual distribution satisfactorily (Table II). The chance probability of obtaining so poor a fit if the distribution was in fact Poisson or negative binomial was less than .05 on all blocks; therefore the hypothesis of a Poisson or negative binomial distribution was rejected.

TABLE II
Neyman's Distribution Compared to the Actual Distribution of *Ips pini* Strikes per Linear Foot of Slash in the 3.5 – 3.9 inch Diameter Class.

Block	Distribution	Pieces of Slash	Frequency of Strikes							Remainder	Deviation^2 Expected	Degrees of Freedom	Probability
			0	1	2	3	4	5	6				
I.....	Actual	34	11	3	4	2	1	4	1	8	3.98	4	0.42
	Neyman's		10.6	2.1	3.2	3.5	3.1	2.6	2.1	6.3			
II.....	Actual	33	12	8	6	2	0	3	1	1	5.75	4	0.22
	Neyman's		13.1	5.9	5.2	3.6	2.2	1.4	0.8	0.7			
IV.....	Actual	39	31	3	1	0	3	1	0	0	8.81	4	0.07
	Neyman's		29.6	3.2	2.8	1.8	0.9	0.4	0	0.3			
VI.....	Actual	43	22	2	5	5	3	4	0	2	5.01	4	0.29
	Neyman's		20.4	4.5	5.2	4.3	3.1	2.0	1.3	2.2			
VIII...	Actual	51	25	4	4	5	7	3	1	2	6.57	4	0.17
	Neyman's		21.6	7.1	7.2	5.5	3.7	2.4	1.5	2.0			

Table II records the actual frequency of attacks or strikes per linear foot of slash in each block and compares this with the theoretical values of an empirical frequency distribution described by Neyman's contagious distribution.

With Neyman's contagious distribution, the expected proportion of units with zero strikes was first calculated. Successive terms were then computed from the preceding ones. The expression for proportion of zeros was:

$$P_o = e^{-m_1 (1-e^{-m_2})}$$

A simplified version for computing the expression for terms other than zero was:

$$P_{n+1} = \left[\frac{m_1 \cdot m_2 \cdot e^{-m_2}}{n+1}\right] \cdot S_{K=o}^{n} \cdot \left[\frac{m_2^K \cdot P_{n-K}}{K!}\right]$$

Population prior to logging.

The early spring cruise (1952) revealed a stand with 1.7% of the trees infested by bark-beetles (Table III).

The six infested trees subjected to stem analyses produced a total of 311 living *Ips pini* of which 295 were adults. The number of living adults obtained from a single tree ranged from 4 to 166, the mean number being 51.8. An estimate of 176.4 bark-beetles per tree was obtained by multiplying the number of bark-beetles per square foot of bark by the total number of square feet of infested bark. The number of bark-beetles in standing trees was estimated to be 1993 per acre. The number of *Ips pini* adults overwintering at the base of infested trees was calculated from 12 duff samples taken below three infested

TABLE III

Cruise results showing number of trees by diameter class infested in 1952, 1953, 1954. (Within the untouched control block).

D.b.h. inches	Number of Stems in original stand/acre	Infested Stems/ acre prior to logging 1952	Infested Stems/acre after logging	
			1953	1954
1	0.2	0	0	0
2	2.1	0	0	0
3	31.4	1.0	2.7	0
4	99.8	2.3	10.0	0
5	156.3	4.7	14.0	0
6	166.4	1.7	4.6	0.7
7	110.8	0.7	4.0	1.3
8	63.6	0.3	0.7	2.0
9	23.7	0	0.7	0
10	9.1	0.3	0	0.7
11	2.4	0	0	0
12	0.9	0.3	0	0
Total	666.7	11.3	36.7	4.7

TABLE IV
The Spring and Fall populations of *Ips pini* (all stages) in slash 1952

Block	Season	Mean per sq. ft. bark	Standard error of mean	Total per acre
I	Spring	36.9	6.8	271,630
	Fall	22.1	6.2	162,361
II	Spring	26.7	17.2	246,162
	Fall	22.6	14.1	203,335
IV	Spring	2.9	1.1	6,813
	Fall	2.2	0.6	5,076
VI	Spring	28.6	10.4	350,973
	Fall	17.5	7.8	214,443
VIII	Spring	17.2	4.2	200,822
	Fall	10.8	4.6	126,521

trees and amounted to 412 per acre. The total endemic population in the stand prior to logging was estimated to be 2405 per acre.

Populations in slash and stumps following logging

The population determined for the two most common scolytids on the experimental area are shown in Tables IV and V.

The mean number of bark-beetles per square foot of bark in the spring of 1953 was calculated to be 0.7 with a standard deviation of 1.04. The duff samples yielded 1.8 per sq. ft. with a standard deviation of 1.4. The total surviving population was estimated to be 18,720 adults per acre.

Table VI summarizes analyses of variance tests carried out for the spring and fall slash sample and the fall stump sample. Because of the large number of zero samples, plot means were analysed rather than single units of sample. A logarithmic transformation was used.

The recovery of parasites and predators in the slash samples was so consistently low that no reliable estimate of the sampling error was obtainable. The numbers per acre, shown in Table VII, were obtained by multiplying

TABLE V
The Fall Populations of *Hylurgops rugipennis* (all stages) in stumps—1952

Block	Mean per Stump	Standard Error of mean	Total per acre
I	53.4	16.1	12,280
III	50.6	12.2	14,421
IV	157.5	23.6	29,137
VI	31.2	18.7	12,219
VIII	35.1	12.8	12,706

TABLE VI
Summary of Analysis of Variance Tests in Bark-beetle Populations

Sample	Source of Variation	D.f.	Mean Square	F.
Spring Sample.....	Between Blocks	4	1.7467	8.34**
Ips pini.....	Within Blocks	45	0.2093	
Fall Sample.....	Between Blocks	4	1.0039	4.24**
Ips pini.....	Within Blocks	45	0.2367	
Fall Sample.....	Between Blocks	4	0.7607	6.93**
H. rugipennis.....	Within Blocks	45	0.1097	

total bark surface per acre per block by the number of insects per square foot of sample in each block.

Environmental factors and effect on distribution of strikes

Results from the subcortical temperature investigations are presented in Tables VIII to XI. The mean subcortical temperatures on two aspects of slash lying on the ground, and two aspects of slash placed 18 inches above the ground are shown in Table VIII. It was found that the direction the slash lay had no effect on subcortical temperatures of the top aspect or the bottom aspect. For this reason, in Table VIII, readings from top and bottom aspects of slash lying in an east-west direction have been combined with readings from top and bottom aspects of slash lying in a north-south direction.

TABLE VII
Population estimates of Diptera, Hymenoptera and Cleridae in Slash – 1952

Block	Season	Parasites		Predators			
		Hymenoptera		Diptera		Cleridae	
		per sq. ft. bark	per acre	per sq. ft. bark	per acre	per sq. ft. bark	per acre
I.....	Spring	1.30	9559	0.30	2206	0.62	4559
	Fall	2.30	16912	1.10	8088	0.30	2206
II.....	Spring	0.80	7367	0.04	368	0.11	1013
	Fall	0.90	8288	0.70	6446	0.07	645
IV.....	Spring	0.30	691	0.08	184	0.07	161
	Fall	0.10	230	0.16	368	0.08	184
VI.....	Spring	1.20	14747	0.41	5038	0.14	1720
	Fall	0.20	2458	0.57	7005	0.09	1106
VIII.....	Spring	0.50	5842	0.06	701	0.15	1752
	Fall	0.08	935	0.23	2687	0.05	350

TABLE VIII
Mean Subcortical Temperatures of Aerial and Ground Slash in an Exposed Location at Air Temperatures of 23° – 25°C.

	Aerial Slash		Ground Slash	
	Top Aspect	Bottom Aspect	Top Aspect	Bottom Aspect
Mean Temp. (C°)	44.6	27.5	47.5	22.0
Standard Deviation	4.8	3.4	6.6	4.1
Number of readings	12	12	12	12

The data shown in Table IX illustrate the differences between the mean subcortical temperatures on various aspects of aerial slash and ground slash. The subcortical temperature readings from the top and bottom aspects are shown in Table VIII.

TABLE IX
Mean Subcortical Temperatures of Aerial Slash and Ground Slash at Air Temperatures of 23° — 25°C. in an Exposed Location.

Direction of Main Axis	Aerial Slash				Ground Slash			
	East-West		North-South		East-West		North-South	
Aspect on Slash	North	South	East	West	North	South	East	West
Mean Temperature (C°)	27.0	45.5	32.3	34.9	27.7	51.7	34.5	46.7
Standard Deviation	3.1	4.8	4.1	3.7	4.1	3.3	6.1	7.6
Number of readings	6	6	6	6	6	6	6	6

Mean subcortical temperatures for slash lying in an east-west direction and in a shaded location are shown in Table X.

TABLE X
Mean subcortical temperatures of aerial and ground slash lying in an east-west direction at air temperatures of 23° – 24°C. (In shade)

Aspect on slash	Aerial Slash		Ground Slash	
	Top	Bottom	Top	Bottom
Mean temperature (C°)	23.5	21.3	24.5	15.9
Standard deviation	0.4	0.4	1.9	2.0
Number of readings	8	8	8	8

TABLE XI
Mean number of *Ips pini* strikes on various aspects of slash in a shaded block and an exposed block

Aspect on slash	Block I (Shaded)				Block VI (Exposed)			
	Top	Bottom	North	South	Top	Bottom	North	South
Mean no. of strikes	0.9	1.0	0.8	1.1	0.2	0.8	1.0	0.4
Standard deviation	1.2	1.2	1.1	1.0	0.5	1.2	1.1	0.7
Number of pieces of slash	27	27	27	27	36	36	36	36

The data in Table XI represent the mean number of strikes by adults of *Ips pini* per linear foot of slash in the 3.0-3.9 inch diameter class. No significant difference in susceptibility to attack was apparent by t-test between slash lying in an east-west direction and slash lying in a north-south direction. Data for Table XI were taken from the two blocks which showed the greatest contrast in amount of shade. Slash in Block I received considerable shade from the residual overstory. Slash in Block VI was exposed to direct solar radiation. The slash was aerial and lay in an east-west direction.

There were not sufficient pieces of slash lying on the ground to obtain figures for comparison with aerial infested slash.

The slash samples in Blocks I and VI were separated into two types, depending on the character of the bark surface. Type A consisted of slash having curled scales. Type B included slash with no scales or scales closely appressed. Table XII summarizes the slash samples within the 3.0-3.9 inch diameter class and relates the number of attacks by *Ips pini* adults to the type of bark. In Block I the differences were not significant while in Block VI the differences were significant to the 1% level.

Discussion

Distribution in slash

Large-diameter slash appears to be more susceptible to bark-beetle attack than that of small diameter (Table I). Three empirical distributions were tested for conformity with the actual frequency distribution of strikes, within

TABLE XII
Type of bark and frequency of strikes by *Ips pini* adults

Block		Type A with curled scales	Type B with no scales or scales tightly appressed
I (Shaded)	Mean no. of strikes	3.8	4.3
	Standard deviation	3.5	3.5
	Number of pieces	24	27
VI (Exposed)	Mean no. of strikes	3.0	1.2
	Standard deviation	2.9	1.7
	Number of pieces	40	21

a single diameter class. It is realized that the results of the frequency tests, as described in Table II, need to be interpreted with some reservation. This applies in particular to Blocks I and IV where all but one of the frequencies are less than five. The Poisson and the negative binomial distributions both gave probability values of less than .05 in all five blocks. Neyman's contagious distribution gave consistently greater conformity with the actual distribution (Table II). The contagious aspect of this type of distribution implies that where one insect is found, there is a likelihood that some more will be found. A successful attack by *Ips pini* indicates that a piece of slash has satisfied the bark-beetle requirements and is suitable for the establishment of a gallery. It follows that the requirements for other bark beetles will be similarly satisfied by that piece of slash, or an adjacent piece with the same favourable features. If all slash in the 3.5 to 3.9 inch diameter class had satisfied the bark-beetle requirements equally well, then it is assumed the selection of sites for brood establishment would have been a random selection which would have approached a Poisson distribution. The character of the bark and subcortical temperatures influenced selection but only in the exposed slash of Blocks VI and VIII.

A number of workers have drawn attention to the great differences in temperature which may occur between ambient air and subcortical regions of the bark. Many of these workers have also illustrated the effect of high subcortcial temperatures on the behaviour of bark beetles. Graham (1924) illustrated a relationship between subcortical temperatures and the distribution of certain bark and wood inhabiting insects on various aspects of slash. The same author (1920) observed mortality to occur in broods of *Ips pini* when subcortical temperatures reached 112°F. (44.5°C.). Complete mortality of the broods occurred when subcortical temperatures reached 122°F. (50°C.). Somewhat similar temperatures were found to be fatal to broods of *Dendroctonus monticolae* Hopk. (Patterson 1930), *D. brevicomis* Lec. (Miller 1931), *D. frontalis* Zimm. (Beal 1933). Results from the subcortical temperature investigations on the experimental area can be summarized briefly as follows: subcortical temperatures vary according to aspect and degree of exposure (Tables VIII and IX); subcortical temperatures of slash in shaded locations tend to follow the air temperature (Table X); aspects of slash exhibiting high subcortical temperatures due to exposure to direct solar radiation are the least attractive to adults of *Ips pini* (Table XI). In exposed locations, such as Blocks VI and VIII, subcortical temperatures in many instances were above the minimum lethal temperature and considerable mortality within the broods occurred.

Populations

The number of bark beetles per acre which became established in the fresh slash far exceeded the numbers present in the immediate area prior to the logging operation. There were no indications in the field that these new populations were the result of mass invasions from distant sources, but appeared rather to be an infiltration from the stand surrounding the experimental area. Although there were variations in the density of attack per square foot of bark between some blocks, populations on an acre basis were somewhat similar (Table IV). The outstanding exception to this occurred in the diameter limit cut on Block IV. Fewer bark beetles were attracted to this block because of the limited quantity of slash present. Large volumes of fresh slash may vary considerably but attract about equal numbers of bark beetles regardless of the degree of exposure of the slash. New populations of *Hylurgops rugipennis* were fairly constant throughout the cutting blocks with the exception of the populations in Block IV (Table V). The large numbers in this block

are attributed to the stump size resulting from the diameter limit cut. (The mean stump diameter for each block was: I –7.4 ins., II – 7.7 ins., IV – 9.0 ins., VI – 7.7 ins., VIII – 8.2 ins.)

Scolytid populations in all blocks were lower in the fall; factors contributing to the reductions varied. Bark-beetle populations in Blocks I, VI, and VIII were from 37 per cent to 40 per cent lower in the fall than in the spring. In Block I, the slash received considerable shade from the overstory and hence subcortical temperatures remained low. A relatively high population of natural enemies (Table VII) became established and flourished in this block. The reduction in bark-beetle population is therefore attributed largely to the activity of the parasites and predators.

In the exposed blocks VI and VIII, high lethal subcortical temperatures occurred on various aspects of the slash. If adults had not concentrated their attacks on aspects of slash least exposed to direct solar radiation, mortality in these exposed blocks would have been considerably higher. Parasite and predator populations were low. Most of the mortality in blocks VI and VIII was due in large part to high subcortical temperatures.

The low brood reduction in Block II appeared to be a result of the low parasite and predator population and the absence of high subcortical temperatures. A somewhat similar condition prevailed in Block IV.

An estimate of 88.6 per cent mortality in Block I was obtained from slash sample data collected in the early spring of 1953. The surviving 1953 population was approximately 7 per cent of the population which had established itself in this block during the spring and early summer of 1952. Although the number of surviving adults was considerably larger than before the logging operation they did not affect the residual stand.

From the silvicultural standpoint and with reference to lodgepole pine stands of this type existing on the east slope of the Rocky Mountains, the following conclusions appear to be justified:—

1. Cutting to a diameter limit of 6.5 inches in this type of stand does not produce enough slash suitable for bark-beetle increase to constitute any serious hazard.
2. Clear cutting or treatments approaching this, resulting in large volumes of slash, create the greatest bark-beetle hazard. Although solar radiation causes high mortality on exposed aspects, the beetles most frequently select unexposed parts of the slash. Moreover, environmental conditions on clear-cut areas are not conducive to the population build-up of bark-beetle parasites and predators.
3. Selection cut systems resulting in large volumes of slash but retaining a high percentage of shade from the residual canopy, produce an abundance of bark-beetles which are not appreciably reduced by solar radiation. However, environmental factors on such areas favour the build-up of bark-beetle parasites and predators, thereby reducing the hazard to residual stands.

Summary

1. This investigation was carried out in the Federal Forestry experimental area 20 miles southwest of Rocky Mountain House, Alberta. A number of 10-acre blocks of lodgepole pine were logged under different silvicultural methods. Scolytid populations in the standing trees prior to logging were estimated. Scolytid populations in the accumulated slash following the logging operations were estimated.

2. Large diameter slash was more susceptible to attack by *Ips pini* adults than small diameter slash.
3. The distribution of attacks of *Ips pini* adults on slash within a single diameter class was best described by Neyman's contagious distribution. Three empirical distributions were tested: Poisson, negative binomial, and Neyman's contagious.
4. In subcortical regions of slash subjected to direct solar radiation there were temperature differences between aspects of the same piece of slash and between the same aspects of ground slash and aerial slash.
5. Adults of *Ips pini* more commonly attacked portions of exposed slash which were least subjected to direct solar radiation. Attacks were distributed evenly over single pieces of slash which were shaded from direct solar radiation.
6. Subcortical temperatures of shaded slash were slightly lower than air temperatures.
7. The direction in which the main axis of the slash lay did not affect susceptibility of that slash to bark-beetle attack.
8. In exposed locations slash having rough scaly bark was more susceptible to bark-beetle attack than slash having smooth bark with few curled scales. This relationship was not apparent in slash shaded from direct solar radiation.
9. Large volumes of slash attracted about equal numbers of *Ips pini* adults despite varying degrees of shade.
10. Summer mortality of broods in exposed slash was attributed to high subcortical temperatures. Broods in exposed slash did not support as large populations of predators and parasites as broods in shaded slash. Summer mortality of broods in shaded slash was attributed to large predator and parasite populations.
11. The spring (1953) population of bark-beetles which survived the overwintering period was considerably larger than the population which existed on the experimental area prior to logging. The spring (1953) population was too low however to constitute a hazard to the residual stand.

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Plastic Rearing Cage for Maintaining Fresh Conifer Foliage for Insect Rearing[1]

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During the course of control investigations on the black-headed budworm, *Acleris variana* (Fern.), a preliminary difficulty occurred in the mass rearing of budworm larvae under laboratory conditions. In the forest the eggs are laid on the needles of hemlock and balsam fir trees in late summer. The young larvae emerge the following spring and migrate to the growing buds where they commence active feeding. Under laboratory conditions, however, cut hemlock twigs shed their needles in a very few days and thus the budworm eggs are shed with the needles and exposed to desiccation.

This paper describes the growth cages used for the successful maintenance of conifer foliage in the rearing of *A. variana* from eggs to adults under laboratory conditions. The method resulted in the retention of hemlock needles, *Tsuga heterophylla* (Raf.) Sarg., for periods up to six weeks. Tests with white spruce, *Picea glauca* (Moench) Voss, balsam fir, *Abies balsamea* (L.) Mill, Scots pine, *Pinus sylvestris* (L.), red pine, *Pinus resinosa* Ait., eastern hemlock, *Tsuga canadensis* (L.) Carr., and a western fir, *Abies* sp. also proved successful.

The assembled unit as shown in Fig. 1 consists of a water-tight stainless steel tray, a plastic baffle plate, and plastic rearing cages. The stainless steel tray, 24 inches long, 3¼ inches wide, 2½ inches deep is constructed from 26-gauge sheeting with soldered joints. The edge of the tray is double rolled for rigidity. The plastic baffle plate inserts are constructed from ⅛ inch stock plexiglass sheeting. The longitudinal baffles are 23⅞ inches long by 1 inch wide and notched ½ inch deep at 5-inch intervals. The transverse baffle strips are 3 inches by 1 inch with corresponding ½ inch deep notches one-half inch from each end. When assembled, the baffle plate unit should fit freely in the tray since the plastic apparently imbibes water and swells.

The growth cages are constructed from commercially available 6¼ inch by 3 inch by 1⅝ inch transparent plastic boxes.[2] This particular type of box was chosen because of its rigid construction and tight fitting covers. It is thus ideally suited for confining very small insect larvae. The boxes are modified for holding conifer foliage in the following manner: a 5/16 inch square of plastic is cut out mid-point on the rim of the basal end of both the lid and the box. It is important to have these grooves midpoint and matched to allow interchanging of lids and bottoms. Three ½ inch diameter humidity and aeration control holes are cut in the box proper. Two lateral holes are located 2 inches from the basal end; the third is centred in the top. The three holes are offset so that the lid does not interfere with them. All aeration holes are covered on the inside with fine mesh plastic or wire screening.[3] The mesh of the screening is dependent on the size of the insect to be reared.

Assembled, the unit holds 14 plastic cages snugly. A 1-inch gap between the end of the tray and the first cage provides for the addition of water, auxin, or nutrient solution. A cork or plastic block in this gap keeps the cages in position.

1Contribution No. 353, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

2Supplied by R. P. Cargille Laboratories Inc., 117 Liberty St., New York 6, N.Y., U.S.A. (Listed as Model O. Size 6" x 2¾" x 1"). Alternative source: Althor Products, 2301 Benson Ave., Brooklyn, N.J., U.S.A.

3Supplied by Lumite Division, Chicopee Mills, 47 Worth St., New York 13, N.Y., U.S.A. or: B. & S.H. Thompson & Co. Ltd., 651 Notre Dame St. West, Montreal, P.Q.

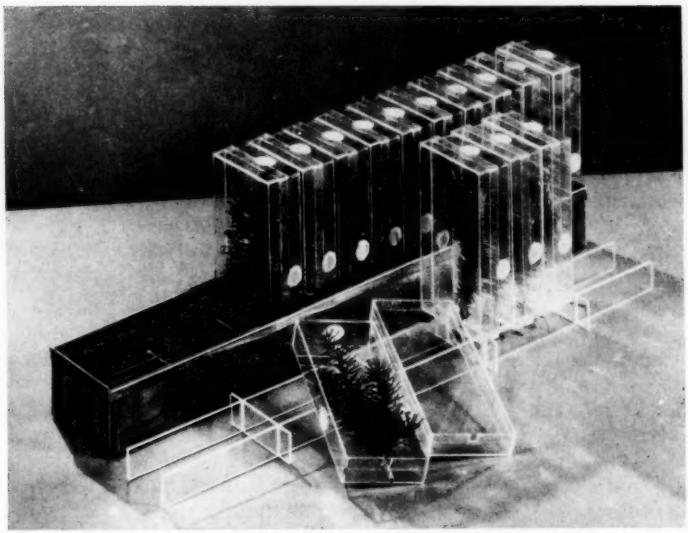

Fig. 1. Rearing tray showing the arrangement of the baffle plate unit and rearing cages with conifer cuttings in place.

There are several advantages in using this particular type of rearing cage as compared with the standard lantern jars. The cages may be used singly or in multiple banks to suit the individual needs of the experiment. Cross-contamination between different cages is greatly reduced since each cage acts as a miniature greenhouse. Inspection of foliage and larvae is possible without disturbing the rearing culture or adjacent material. Lastly, because of its compactness, a relatively large number of insects may be reared in a minimum of space under laboratory or room conditions.

The method has been successfully adapted to different sized cages. Although the present arrangement of the aeration holes has proved most successful, humidity control may be obtained by varying the location and size of the openings.

Since the cages are of a plastic material readily attacked by some common organic solvents and heat, sterilization by these means is not practicable. They are, however, unaffected by methyl or ethyl alcohol, hot soap solutions, and antiseptic rinses.

The author wishes to acknowledge the technical advice and assistance of Mr. C. Jackson, Entomology Workshop, Science Service Bldg., Ottawa.

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Surveys of Parasites of *Hylemya* spp. (Diptera: Anthomyiidae) That Attack Cruciferous Crops in Canada[1]

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Surveys of parasites of species of *Hylemya* that attack cruciferous crops were made in Canada as part of a biological control program against these insects. The most important species are *Hylemya brassicae* (Bouché), *H. floralis* (Fall.), and *H. planipalpis* (Stein.). Though not of primary importance on crucifers, *H. cilicrura* (Rond.) was often associated with the other species. The geographical distributions of these species in Canada were described by Brooks (1951).

Former records of parasites of *Hylemya* spp. in Canada were obtained during investigations on the biology and control of these insects. No comprehensive survey for parasites was attempted previously. Gibson and Treherne (1916) reported the staphylinid *Baryodma ontarionis* Casey and the cynipid *Cothonaspis gillettei* Washb. as parasites of *Hylemya* spp. Fletcher (1901, p. 230) reported the staphylinid *Aleochara nitida* Grav. as abundant at Ottawa, and also reported a cynipid, *Eucoila anthomyiae* Ashm. Schoene (1916) recorded *Aleochara bipustulata* (L.) as the most important staphylinid parasite and also recorded a cynipid, *Pseudoeucoila gillettei* Ashm.

Materials and Methods

Early in the investigations sufficient eggs and larvae were examined to indicate that there are no egg parasites and that those that attack the larvae complete their development in the puparia. Host insects, almost entirely puparia, were collected by officers of the Field Crop Insect Unit and of the Biological Control Unit, Entomology Division, and also by Dr. John Oughton of the Ontario Agricultural College, Guelph. All the material was then reared at Belleville.

On arrival at the laboratory, the puparia were placed singly in one-inch shell vials and kept at approximately 80 per cent relative humidity and 75°F. Vials containing emerged adults were removed daily. Parasites that could not be identified were sent to the Systematic Entomology Unit, Ottawa, for identification. All hosts, both parasitized and unparasitized, were identified from the puparia with the aid of Brooks' (1949, 1951) keys. Representative samples checked by the specialists at Ottawa showed that identifications were being made with a high degree of accuracy.

After hosts and parasites ceased emerging, the remaining puparia were dissected and the contents analysed.

Results and Discussion

The data for the collections are given in Tables I and II. Only two parasites, *Aleochara bilineata* Gyll. (*Staphylinidae*) and *Trybliographa rapae* (Westw.) (Cynipidae), were found throughout Canada and on all four species of *Hylemya*. *A. bipustulata* was found in several areas, usually in small numbers. Not shown in the tables are two parasites: *Phygadeuon* sp. (Ichneumonidae), one specimen of which was reared from *H. cilicrura* from Mount Pearl, Newfoundland; and several specimens of *Aphaereta auripes* (Prov.). (Braconidae), reared from *H. brassicae* from St. Martin and St. Rose, Quebec. These may be considered as occurring only occasionally on *Hylemya* spp.

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TABLE I

Parasites reared at Belleville, Ontario, from *Hylemya* spp. collected in various localities in Canada, 1951

Locality	Number of Puparia	Percentage of puparia belonging to				Percentage parasitized by											
		Hylemya brassicae	*H. cilicrura*	*H. planipalpis*	*H. floralis*	*Alochara bilineata*	*A. bipustulata*	*Trybliographa rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*
						of *H. brassicae*			of *H. cilicrura*			of *H. planipalpis*			of *H. floralis*		
Mount Pearl, Nfld.	515	95.0	5.0			63.0		1.4	42.3								
Charlottetown, P.E.I.	684	94.3	5.7			9.5		.8	2.6								
Hampton, P.E.I.	11	100.0						9.1									
Port Williams, N.S.	560	96.1	3.9			13.2		1.5									
Sheffield Mills, N.S.	268	99.3	.7			12.8		43.2									
Maugerville, N.B.	1229	95.9	4.1			7.7		8.5	3.9		5.8						
St. Martin, Que.	35	94.3	5.7			48.5											
St. Geneviève, Que.	303	100.0				52.5	.7	4.6									
St. Dorothee, Que.	211	91.9	8.1			35.6		.5	17.6		11.8						
St. Foy, Que.	720	94.2	5.8			14.0	.3	3.7	11.9		7.1						
St. Cyrille, Que.	139	87.1	12.9			5.0		1.7	5.6								
Ile Bizard, Que.	61	100.0				42.6		24.6									
St. Cesaire, Que.	196	99.5	.5					10.8									
Merivale, Ont.	277	87.7	12.3			5.3		.4			26.5						
Huff's Island, Ont.	187	82.4	17.6			23.4		.6			6.1						
Sidney Twp., Ont.	92	92.4	7.6			1.2											
Guelph, Ont.	266	63.2	36.8			7.8	8.3	.5	2.0	14.3	3.1						
Dauphin, Man.	128		3.1	.8	96.1						25.0				28.5		
Brandon, Man.	929		22.5	6.0	71.5				4.8	.5	3.4	19.5		1.9	1.1	.3	.2
Saskatoon, Sask.	340		26.7	2.1	71.2						45.1				1.2		
Broxburn, Alta.	74	86.5	13.5			18.8	1.6		30.0								
Abbotsford, B.C.	361	95.6	4.4			50.7			37.5								
Kamloops, B.C.	114	50.9	49.1			12.1		1.7	17.9		8.9						
Agassiz, B.C.	54	87.0	13.0			46.8			42.9								
Chilliwack, B.C.	95	98.9	1.1			29.8											
Cloverdale, B.C.	489	54.0	46.0			13.2			12.4								

TABLE II

Parasites reared at Belleville, Ontario, from *Hylemya* spp. collected in various localities in Canada, 1952 and 1953

Locality	Number of Puparia	Percentage of puparia belonging to				Percentage parasitized by											
		Hylemya brassicae	*H. cilicrura*	*H. planipalpis*	*H. floralis*	*Aleochara bilineata*	*A. bipustulata*	*Trybliographa rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*	*A. bilineata*	*A. bipustulata*	*T. rapae*
						of *H. brassicae*			of *H. cilicrura*			of *H. planipalpis*			of *H. floralis*		
1952																	
Long Pond, Nfld.	101	96.0	4.0			45.4											
Goulds, Nfld.	238	87.8	12.2			33.0		4.3	10.3		6.9						
Charlottetown, P.E.I.	70	57.1	42.9														
Kentville, N.S.	454	96.3	3.7			8.4		16.5	11.8		17.6						
Sheffield Mills, N.S.	246	98.4	1.6			10.7		46.3			50.0						
St. Martin, Que.	150	96.0	4.0			2.1											
Charlesbourg, Que.	100	98.0	2.0			51.0		2.0									
St. Cesaire, Que.	92	97.8	2.2			37.8		2.2	50.0		50.0						
St. Rose, Que.	76	89.5	10.5														
Dauphin, Man.	13		7.7		92.3				100.0						33.3		
Brandon, Man.	923		5.1	.2	94.7				4.3		6.4				14.9		
Simpkins, Sask.	88		5.7	2.3	92.0						20.0				1.2		
Kamloops, B.C.	254	89.4	10.6								3.7						
Cordova, B.C.	124	100.0				1.6											
1953																	
St. John's, Nfld.	994	98.6	1.4			4.7		10.7	7.1								
Charlottetown, P.E.I.	523	99.4	.6			.2		4.0									
Sheffield Mills, N.S.	866	98.4	1.6			2.7		35.0	14.3		28.6						
Brandon, Man.	402		2.2		97.8						33.3				16.3		.3

Wide variations in the abundance of the two major parasites are apparent. Data on such factors as season at which collections were made, type of soil, and crop, which might influence abundance, were not sufficient to warrant an attempt to associate these factors with percentage of parasitization.

As the cynipid *T. rapae* attacks the early instars and emerges from the puparia, a representative sample of puparia gives a representative sample of parasitization by this species. The two staphylinids, *Aleochara* spp., attack the puparia (Colhoun, 1953), which are vulnerable to attack for the greater part of the time they are in the soil. The degree of parasitization by this species depends, therefore, on the length of time the puparia are in the soil before they are collected. No way was found to determine at what ages the samples of puparia were collected. Hence, the data are probably accurate for parasitization by the cynipid but, to varying degrees, below the actual for the staphylinids.

The percentage of *H. floralis* parasitized by *T. rapae* was much lower than that found for the same species in Europe, where, in collections made by officers of the Biological Control Unit, it was equal to or higher than parasitization of *H. brassicae*. In Europe *H. brassicae* and *H. floralis* occur together and *H. brassicae* provides host larvae at the right stage for parasitization when *T. rapae* emerges in the spring. *H. floralis* larvae appear much later and are attacked by *T. rapae* that have gone through one generation on *H. brassicae*. In Manitoba and Saskatchewan, where *H. brassicae* does not occur, *H. planipalpis* is an early-season host but is present in small numbers and does not support a sufficient first-generation population of *T. rapae* to produce a high percentage of parasitization on *H. floralis*. Collections received from Dr. Satoru Kuwayama, Hokkaido, Japan, were entirely of *H. floralis* and did not contain *T. rapae* due, possibly, to the lack of a population of *H. brassicae* to support the parasite through the first generation.

Comparison by Mr. W. J. Brown (in litt.), Insect Systematics and Biological Control Unit, Ottawa, of a large number of specimens of *B. ontarionis* and *A. bilineata* reared from *H. brassicae* puparia from Europe showed they are conspecific. It is probable, therefore, that, because this species is much more abundant than *A. bipustulata*, most, if not all, the earlier workers in North America who referred to staphylinid parasites under other names were referring to *A. bilineata*. It was possible in one instance to prove that this was so. Burks (1952, pp. 379-380) referred to a staphylinid parasite of *H. brassicae* as *Aleochara bimaculata* Grav. in an account based on work done in Illinois in 1938 or 1939. Specimens collected at that time were found in the collection of the Illinois Natural History Survey. When these were compared by Dr. M. J. Sanderson (in litt.), Illinois Natural History Survey, Urbana, Illinois, they were found to be of that species.

A. bipustulata is a smaller species than *A. bilineata* and is found more frequently on *H. cilicrura* than on *H. brassicae*. Its life-history in the laboratory is similar to that of *A. bilineata* except that the larvae find difficulty in entering large or thick-skinned puparia of *H. brassicae*. This may be why the species is found more often on *H. cilicrura*, which, as a rule, has a relatively light-skinned puparium. It is rarely found on *H. floralis*, the largest of the four species.

There was some morphological variation in the parasites that are listed here as *T. rapae*. Most of those reared from *H. brassicae* and *H. floralis* had the cubital vein present as a brown line extending to the apex of the fore wing, whereas in those reared from *H. cilicrura* this vein was absent. The latter specimens also had the legs blackish, rather than reddish as in those reared from the

two larger species. These characters may possibly be influenced by nutrition but, until there is further evidence, Dr. O. Peck (in litt.), Insect Systematics and Biological Control Unit, Ottawa, considered all the specimens as of *T. rapae*. It can be assumed that *Cothonaspis gillettei* (Gibson and Treherne, 1916), *Eucoila anthomyiae* (Fletcher, 1901), and *Pseudoeucoila gillettei* (Schoene, 1916) were this species since no other cynipid parasites were taken in the extensive collections made in this study. Notes on the life-history and habits of this species and additional distribution records were given previously (Wishart and Monteith, 1954).

Acknowledgments

The contribution to this project by officers of the Systematic Entomology and Biological Control Unit, Entomology Division, Ottawa, is gratefully acknowledged. Particular mention must be made of the assistance given by Mr. W. J. Brown, Dr. Oswald Peck, Mr. G. S. Walley, and Dr. Wm. R. M. Mason in identifying parasites, and by Mr. G. E. Shewell, Mr. J. G. Chillcott, and Mr. J. F. McAlpine in identifying hosts. Several members of the Belleville staff, notably Dr. E. H. Colhoun and Miss Elizabeth Monteith, made significant contributions to the laboratory work involved. Perhaps the greatest debt is to the many officers of other units of the Entomology Division who took time from their own work to make the collections on which most of the data are based.

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A Trap for Insects Emerging from the Soil[1]

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The larch sawfly, *Pristiphora erichsonii* (Htg.), overwinters in a cocoon in the ground and has an adult emergence period that may last two months. Ecological studies of this insect require information on the number of adults emerging and the seasonal pattern of emergence. Several types of open-bottomed screen emergence cages without traps were tested but the insects caught in such cages were difficult to remove. Also, many of the adults were lost in the vegetation unless the cages were visited more frequently than was practicable. The trap described here, attached to a screen cage, eliminates the objections given above and could be of use to persons interested in insects which emerge from the soil.

Fig. 1 shows the emergence cage and trap in use. The trap is secured to the top of a pyramidal screen cage attached to a frame of 1- by 2-inch lumber covering an area of 2 square feet. Stove wire is used to tighten the screen around the trap and to prevent escape of insects. The construction of the trap (Fig. 2) requires a round plastic container with a double sealing ethylene cover[2], and a clear 8 oz. styrene funnel[3]. The bottom is removed from the container and the shaft from the funnel. A 1¼ inch hole is cut in the lid of the container and closed with No. 40 copper screening. The funnel is fixed inside the container (Fig. 2) by soaking the contact surfaces in benzene for several minutes and then pressing the two surfaces together. The outside of the container is painted with

1Contribution No. 352, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.
2Catalog No. 1352, Series C., Rogers Plastics Limited, Rawdon, Quebec. Diameter 3¾ inches, height 3⅛ inches.
3Stock No. BW82, Molded Plastics, 1346 East Walnut Street, Pasadena, California, U.S.A.

Fig. 1. Insect trap wired to screen emergence cage.

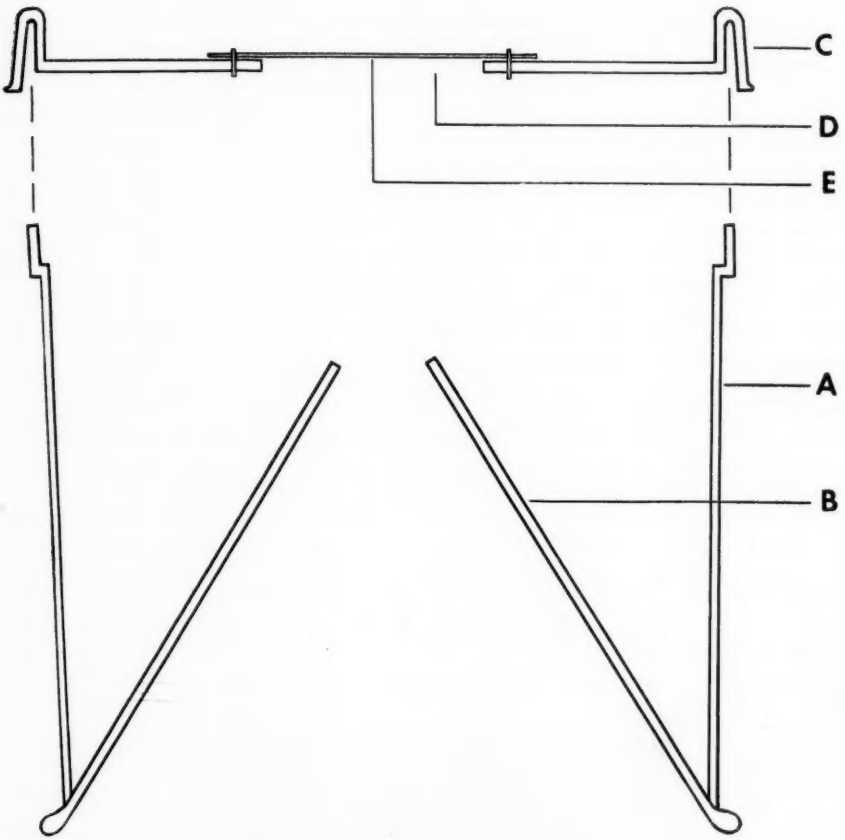

Fig. 2. Cross-section of insect trap showing component parts:
A. Plastic container with bottom removed
B. Plastic funnel with shaft removed
C. Polyethylene lid
D. 1¼" hole
E. No. 40 copper screen

black Duco enamel and the lower surface of the funnel roughened with emery paper. When the trap is placed in the field, a small amount of "Tanglefoot" is spread on the inner wall of the container and the upper surface of the funnel.

Newly emerged insects climbed or flew to the top of the screen, and thence through the funnel, attracted by the light coming through the hole in the lid. Once inside the trap they became entangled on the sticky surface. The traps were visited every four days to record and remove the trapped insects. A few adult larch sawflies caught in the seams of the screen were recorded. The trap also caught other Hymenoptera, many Diptera, and some Coleoptera, Trichoptera, and Lepidoptera. Laboratory experiments with 190 larch sawfly adults, placed in the cages in groups of 20 to 40, showed that 90 to 95 per cent of the insects were caught in the trap.

(Received January 31, 1957)

Factors in Resistance of Peas to the Pea Aphid, *Acyrthosiphon pisum* (Harr.) (Homoptera: Aphididae). II. Amino Acids[1]

By J. L. Auclair[2], J. B. Maltais[2], and J. J. Cartier[3]
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In field investigations on the relative resistance of varieties of peas, *Pisum sativum* L., to the pea aphid, *Acyrthosiphon pisum* (Harr.), the average number of aphids per terminal growth for 13 years (Maltais, 1937, 1950, 1951, and unpublished technical report, 1950-54) for six varieties was as follows: Perfection, 39.6; Daisy, 32.6; Lincoln, 35.0; Laurier (H-103), 9.8; Champion of England, 11.8; and Melting Sugar, 16.8. In a preliminary investigation by Auclair and Maltais (1950), 11 free amino acids were detected in pea plant extracts by paper chromatography. From a visual comparison of chromatograms, the variety Perfection appeared to contain a higher concentration of most free amino acids than the variety Laurier. This is a report on the quantitative estimation of the free and total amino acid contents of the three susceptible varieties (Perfection, Daisy, and Lincoln) and the three resistant varieties (Laurier, Champion of England, and Melting Sugar) by the method of paper chromatography.

Methods

The methods for the growing of peas in the field and the greenhouse, the standardization of plant growth stages and plant sampling, and the initial processing of fresh and dried plant samples were described by Maltais and Auclair (1957).

Preparation of Samples for Chromatographic Analysis

Determination of Free Amino Acids.—Amounts of free amino acids were determined from 1-, 2-, and 5-ml. water-extract samples preserved dried in round-bottom micro-beakers in a desiccator. The dry samples were redissolved with acidulated water to one-tenth of the original volume and aliquots chromatographed. Results are expressed in micrograms per hundred microlitres of the original water extract.

Determination of Total Amino Acids.—The plant samples were hydrolyzed as follows:

Dry matter: Fifty milligrams of dry matter and 1 ml. of 6 normal hydrochloric acid were placed in a 3-ml. Pyrex test tube. The tube was sealed under oxygen flame and placed for 12 hours in the autoclave at 15 pounds' pressure and 120°C. After hydrolysis, the seal was broken and the tube placed under vacuum over a mixture of sodium hydroxide pellets and granular anhydrous calcium chloride until the hydrolysate was dry. It was then redissolved with water to one-half of the original volume and aliquots chromatographed. Results are expressed in micrograms per milligram of dry matter.

Water extracts:—One millilitre of water extract was concentrated by evaporation under vacuum to about 0.25 ml. and mixed with 0.25 ml. of 6 normal hydrochloric acid in a 3-ml. Pyrex test tube. Hydrolysis was carried out as described for dry matter. The dry hydrolysate was redissolved with water to the original volume and aliquots chromatographed. Results are expressed in micrograms per hundred microlitres of the original water extract.

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Analysis by Paper Chromatography

The method of paper chromatography developed by Consden, Gordon, and Martin (1944), and modified by capillary ascent by Williams and Kirby (1948), was used with slight modifications as follows: Five- and ten-gallon earthenware jars covered with glass plates served as chromatograph chambers. Large filter paper sheets (46.5 cm. by 57 cm.), Whatman No. 1, were cut in four equal pieces for two-dimensional chromatography. The solvent mixtures were prepared as described by Auclair and Maltais (1954). The chromatography room was kept at a temperature of $78\pm2°$F. and a relative humidity of 80 ± 5 per cent and the solvent runs were carried out during a constant period of time. The chromatograms were dried overnight at room temperature after each run.

For the qualitative determinations, a number of tests described by Dent (1948), Crumpler and Dent (1949), Block *et al* (1952), Auclair and Maltais (1952), and Miettinen *et al.* (1953) were made to confirm the identity of the amino acid spots occurring regularly on the chromatograms.

Three quantitative methods of paper chromatography were used.

1. That of Auclair and Dubreuil (1952).

2. That of Block (1950). In this method, the maximum colour density of amino acid spots is determined with a photo-electric densitometer. Details of the method as applied in the present work are as follows: After the solvent runs are completed, the dried chromatograms are dipped into a solution of ninhydrin (0.1 per cent in *n*-butanol), allowed to dry for five minutes after dipping is completed, developed in the oven at 60°C. for 30 minutes, and stored in the dark. The densitometer readings are made 20 hours later with a Welch Densichron Transmission Densitometer with blue probe and a log scale for optical density. The apparatus is standardized as follows: With a 3-mm. aperture on the light transmission unit, the blue probe is lowered, the light (without filter) is focused to give maximum intensity, by means of the light focusing mechanism and the rheostat, and the galvanometer is adjusted to zero reading. With the calibrated step wedge (No. 1834) at No. 4 (giving a density reading of 0.40 on the galvanometer), 0.34 is subtracted from the galvanometer, leaving a reading of 0.06. The value 0.34 represents the optical density of the white paper and is used as a constant blank. The Densichron is then ready for optical density determinations on the chromatograms. For the amino acid proline, the above procedure is slightly modified in that a Wratten filter (blue C4) is used and 0.40 is subtracted from the galvanometer reading.

3. A combination of those of Block (1950) and Thompson *et al.* (1951). Block's method was slightly modified by carrying the ninhydrin reaction in a special cabinet containing carbon dioxide saturated with *n*-butanol vapours, at 60°C. for 30 minutes. This modification in the ninhydrin development procedure was adapted from a quantitative method described by Thompson *et al.* (1951).

Author—Auclair.

Tables I to IV show that 14 to 25 amino acids and amides were identified in the water extracts and the dry samples of the terminal and middle growths of the pea plants. In that group are included the common amino acids recognized as "building blocks" of the protein molecule, the two amides asparagine and glutamine, and some amino acids not recognized as part of the protein molecule such as beta-alanine, gamma-amino-butyric acid, and homoserine. It is very probable that both leucine and isoleucine were present in all the samples analyzed. It is probable that methionine and cysteine are present in the pea plant and are partly or wholly oxidized to methionine sulfoxide and cysteic acid either during the extraction of the plant samples or during chromatography. There seemed

to be no qualitative differences in the amino acids and amides found in the six varieties of peas analyzed. Five unknown compounds were detected and they were named according to their positions on the two-dimensional chromatograms (Tables I and II).

The three susceptible varieties contained higher concentrations of free and total amino acids and amides than the three resistant ones. The quantitative differences were consistent for most amino acids and in all the totals in each table. Student's *t* test showed significant differences at the five per cent level between the totals (Tables I-III) obtained from the water extract of the suscept-

TABLE I

Free amino acids (micrograms per 100 microlitres of water extract) and total amino acids (micrograms per 100 microlitres of hydrolyzed water extract) in terminal growth samples of a susceptible and a resistant variety of pea at three stages of growth, from field and greenhouse

Amino acid	Perfection (susceptible)				Laurier (resistant)			
	Field			Greenhouse	Field			Greenhouse
	Early growth	Full bloom	Full bloom	First blossom	Early growth	Full bloom	Full bloom	First blossom
	Water extracts*		Hydrolyzed water extracts**	Water extracts*	Water extracts*		Hydrolyzed water extracts**	Water extracts*
α-Alanine	12.0	10.0	17.4	16.7	12.0	12.0	26.4	16.7
β-Alanine	3.0	3.7			3.7	1.7		
γ-Amino-butyric acid	55.0	27.5	58.3	27.5	55.0	22.0	23.7	36.7
Arginine	20.0	40.0	6.7	48.0	12.0	15.0	2.7	24.0
Asparagine	67.5	60.0		36.0	60.0	21.6		18.0
Aspartic acid	14.0	14.0	89.8	11.7	7.0	2.8	57.1	3.1
Cysteic acid	0.3	0.5		0.2	0.3	0.3		0.1
Glutamic acid	15.0	8.6	20.0	15.0	15.0	12.0	16.0	15.0
Glutamine	16.7	15.0		12.5	16.7	6.0		10.0
Glycine	2.0	3.0	6.8	3.0	2.0	1.2	7.3	1.5
Leucine and/or isoleucine	8.3	6.7	13.3	7.1	5.0	6.2	13.1	5.3
Lysine	15.9	6.7	94.3	20.0	10.0	9.1	73.1	7.7
Methionine sulfoxide	trace	trace			trace	trace		
Phenylalanine	trace	trace			trace	trace		
Proline	2.4	8.6	4.7	5.0	5.0	5.4	4.7	4.1
Serine	7.8	7.8	13.5	7.0	7.8	4.7	8.4	5.0
Threonine	15.0	15.0	10.8	12.0	10.0	6.0	8.4	6.0
Tryptophan	4.4	4.9		12.2	4.4	3.1		6.3
Tyrosine	8.3	5.0	2.9	6.2	6.7	5.9	3.0	4.3
Valine	4.8	6.0	7.1	6.7	3.0	2.4	6.4	3.4
Total	272.4	243.0	345.6	246.8	235.6	137.4	250.3	167.2
Over-alanine***	200				60			
Left-alanine	7	9			8	9		
Right-proline	35	35			25	25		

*Method of analysis by Auclair and Dubreuil (1952).
**Method of analysis by Block (1950).
***Unknown spot with corresponding minimum volume of sample in microlitres at which it was barely detectable.

TABLE II

Free amino acids (micrograms per 100 microlitres of water extract) and total amino acids (micrograms per milligram of dry matter) of terminal growth samples of two susceptible and two resistant varieties of peas at first blossom stage, from greenhouse. Combined methods of analysis: Block (1950) and Thompson *et al.* (1951)

Amino acid	Susceptible				Resistant			
	Perfection		Lincoln		Laurier		Champion of England	
	Water extracts	Dry matter hydrolyzed	Water extracts	Dry matter hydrolyzed	Water extracts	Dry matter hydrolyzed	Water extracts	Dry matter hydrolyzed
α–Alanine	10.3	14.2	7.4	14.0	5.2	11.4	5.7	11.4
γ–Amino-butyric acid	4.2	5.2	6.3	5.5	4.6	5.3	4.1	4.7
Arginine		1.6		1.9		1.5		1.6
Asparagine	32.6		34.4		7.7		19.6	
Aspartic acid	5.0	28.4	5.6	33.5	1.0	10.6	2.6	14.2
Cysteic acid	1.7	1.7	4.0	1.6	1.7	0.1	1.2	0.4
Cystine	1.0	3.3	3.5	4.2	2.8	2.9	1.3	3.1
Glutamic acid	20.0	21.2	18.5	23.3	9.1	18.4	7.9	15.3
Glutamine	6.9		7.6		3.5		5.8	
Glycine	0.8	5.8	0.9	8.7	0.3	5.4	0.8	3.9
Homoserine	23.9	18.4	25.3	17.2	19.7	22.3	31.2	19.2
Leucine and/or isoleucine	14.8	80.1	13.2	76.3	6.2	45.3	8.5	46.2
Lysine	8.8	83.9	7.2	82.2	4.0	39.0	5.0	62.7
Methionine sulfoxide		3.2		3.1		2.0		2.2
and arginine*	3.4		3.6		2.7		2.5	
Phenylalanine	4.2	26.4	3.2	35.6	1.6	28.1	2.4	26.7
Proline	2.6	2.1	2.1	2.0	2.3	1.4	1.7	1.5
Serine	2.8	10.2	3.1	9.7	2.0	5.9	2.8	6.1
Threonine		23.7		19.7		16.4		17.9
Tyrosine	3.1	5.7	2.8	6.5	1.9	4.9	2.3	4.7
Valine	4.4	0.6	4.2	0.6	2.1	0.4	2.8	0.4
Total	150.5	335.7	152.9	345.6	78.4	221.3	108.2	242.2
Over-proline**		0.55		0.61		0.50		0.71
Under-proline		0.56		0.61		0.47		0.52

*Approximately 80 per cent methionine sulfoxide and 20 per cent arginine.
**Unknown compound with corresponding optical densities.

ible and the resistant varieties both at first-blossom and full-bloom stages. Differences were significant at the one per cent level between the totals (Tables II and III) obtained from the dry samples of the susceptible and the resistant varieties. In the four varieties analyzed, the free amino acid content was considerably lower in the middle than in the terminal-growth samples (Table IV). The differences between varieties were found in the plants grown in the field and in the greenhouse, and were of similar magnitude when obtained by any of the three methods of quantitative analysis.

Discussion

The important result consists in significant quantitative differences in the free and total amino acid and amide contents between varieties throughout the

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stages of growth corresponding with the usual period of pea aphid infestation in the field. Aphids growing and reproducing on resistant varieties feed on plant material containing a lower concentration of free and total amino acids than those growing and reproducing on susceptible varieties.

Several workers have shown that a number of amino acids are essential in the diets of various insects for normal growth and development. In Table V are listed seven insect species for which the amino acid requirements have been established; seven to 16 of the 20 amino acids listed are essential in the diet of the insect concerned. The 16 essential amino acids are: arginine, histidine, isoleucine, methionine, tryptophan, and valine (probably essential for the seven species); leucine, lysine (six species); phenylalanine, threonine (five species); glycine (four species); cystine, proline, alanine, serine, and tyrosine (one to three

TABLE III

Free amino acids (micrograms per 100 microlitres of water extract) and total amino acids (micrograms per milligram of dry matter) of terminal growth samples of two susceptible and two resistant varieties of peas at full bloom stage, from field

Amino acid	Susceptible				Resistant			
	Perfection		Daisy		Laurier		Melting Sugar	
	Water extracts*	Dry matter hydrolyzed**	Water extracts*	Dry matter hydrolyzed**	Water extracts*	Dry matter hydrolyzed**	Water extracts*	Dry matter hydrolyzed**
α-Alanine	18.4	23.3	10.4	20.4	1.5	17.8	7.5	13.6
β-Alanine	3.1		2.2		1.7			
α-Amino-butyric acid	0.9				1.0			
γ-Amino-butyric aid	2.1	4.4	8.8	3.9	5.1	3.7	3.7	1.6
Arginine	5.4	34.0	3.0	30.3	3.1	27.1	2.5	20.0
Asparagine	27.5		24.0		15.4		5.2	
Aspartic acid	10.3	39.6	11.8	38.6	1.9	21.5	6.1	22.6
Cysteic acid		1.4		1.7		0.5		0.6
Cystine	0.7		1.7		0.2		0.9	
Glutamic acid	3.1	41.5	3.8	46.0	2.7	29.5	4.7	33.7
Glutamine	15.6		10.3		4.8		7.2	
Glycine	1.4	18.2	1.8	17.0	1.5	12.2	1.1	9.0
Histidine		2.8		2.8		2.0		2.4
Homoserine		8.7		6.6		11.4		6.0
Leucine and/or isoleucine	7.2	76.7	6.0	76.7	5.8	60.7	3.8	50.3
Lysine	10.8	65.8	9.6	56.0	4.8	33.3	6.2	48.5
Methionine sulfoxide	2.6	2.8	2.5	3.1	1.1	2.7	2.5	2.5
Phenylalanine	2.3	23.1	3.6	25.1	2.4	18.3	2.4	16.8
Proline	4.6	12.2	1.2	8.6	2.4	6.7	1.0	6.7
Serine	9.4	16.1	4.8	15.0	3.9	7.7	2.1	7.7
Threonine	7.3	20.2	4.3	17.2	4.7	15.4	3.5	13.4
Tryptophan	1.7		1.1		1.4			
Tyrosine	2.2	5.2	2.2	5.0	2.2	4.4	1.5	4.3
Valine	4.9	28.2	3.2	27.5	3.9	21.0	1.8	19.7
Total	141.5	424.2	116.3	401.5	71.5	295.9	63.7	279.4

*Method of analysis by Block (1950).
**Combined methods of analysis: Block (1950) and Thompson *et al.* (1951).

TABLE IV

Free amino acids (micrograms per 100 microlitres of water extract) in middle growth samples of two susceptible and two resistant varieties of peas at first blossom stage, from greenhouse. Combined methods of analysis: Block (1950) and Thompson *et al.* (1951)

Amino acid	Susceptible		Resistant	
	Perfection	Lincoln	Laurier	Champion of England
α-Alanine	3.2	3.5	1.5	1.9
γ-Amino-butyric acid	3.7	4.5	2.9	2.3
Asparagine	7.3	8.3	3.2	4.0
Aspartic acid	3.8	3.6	2.3	0.9
Cystine	2.0	2.1	1.6	0.9
Glutamic acid	4.6	5.3	2.0	2.2
Glutamine	4.6	4.4	2.8	2.0
Glycine	0.3	0.3	0.2	0.2
Homoserine	14.0	9.9	6.9	10.0
Leucine and/or isoleucine	4.9	7.7	2.0	2.0
Lysine	2.0	2.6	1.5	1.6
Methionine sulfoxide and arginine*	1.4	1.8	0.7	0.7
Phenylalanine	1.7	2.8	0.3	0.4
Proline	1.1	2.4	0.7	0.8
Serine	1.0	1.4	0.9	0.7
Tyrosine	1.3	1.5	0.8	0.7
Valine	1.5	2.4	0.5	0.6
Total	58.4	64.5	30.8	31.9

*Approximately 80 per cent methionine sulfoxide and 20 per cent arginine.

species). Lack of any one of these amino acids reduces growth, inhibits moulting or pupation, or considerably increases mortality.

No adequate data have been published on the minimum quantitative amino acid requirements of insects. Synthetic diets devised for insects generally contain a high percentage of amino acids and proteins. For instance, House (1949a), working with the German cockroach, *Blattella germanica* (L.), devised a suitable chemically defined diet containing 30 per cent amino acid by weight. Lemonde and Bernard (1951a), working with the confused flour beetle, *Tribolium confusum* Duv., made up a synthetic diet containing 20.7 per cent of an amino acid mixture. These authors (1953) determined the minimum protein requirements of the drug-store beetle, *Stegobium paniceum* (L.), at 10 per cent of the diet. When the protein level of the diet fell to 6 per cent, the time required for the larvae to reach the adult stage was more than doubled.

The quantitative differences in amino acid content observed between varieties are apparently factors in resistance or susceptibility to infestation by aphids. Aphids feeding on resistant varieties may not obtain enough of each essential amino acid per unit of time to sustain optimum growth and reproduction. In other words, on varieties low in essential amino acids, aphid growth and reproduction proceed at a lower rate than on varieties high in essential amino acids. This would cause the total aphid population on resistant varieties to be usually smaller than that on susceptible varieties.

TABLE V
Amino acids required in diet by various insects

Amino acid	*Blattella germanica* (L.) House (1949b), Hilchey (1953), Noland and Baumann (1951)	*Tribolium confusum* Duv. Lemonde and Bernard (1951b)	*Oryzaephilus surinamensis* (L.) Davis (1952)	*Attagenus* (?) sp. *Moore* (1946)	*Drosophila melanogaster* Mg. Hinton *et al.* (1951), Lafon (1939), Rudkin *et al.* (1947)	*Aedes aegypti* (L.) Goldberg and De Meillon (1948)	*Pseudosarcophaga affinis* (Fall.) House (1954)	Number of species requiring amino acid in diet
α–Alanine	+*	−	−	−			+?	2
Arginine	+?	+	+	+	+	+	+	7?
Aspartic acid	−	−		−			−	
Cysteine							−	
Cystine	+?	−	−?	−	+	+		3
Glutamic acid	−	−		−			−	
Glycine	−	−	+?	−	+	+	+	4
Histidine	+	+	+?	+	+	+	+	7?
Hydroxyproline	−	−		−			−	
Isoleucine	+	+	+?	+	+	+	+	7?
Leucine	+	+		+	+	+	+	6
Lysine	+	+	−	+	+	+	+	6
Methionine	+	+	+?	+	+	+	+	7?
Phenylalanine	−	+		+	+	+	+	5
Proline	+♂	−	−	−	+?	+	−	3
Serine	+♂	−		−			+?	2
Threonine	−	+		+	+	+	+	5
Tryptophan	+	+	+	+	+	+	+	7
Tyrosine	−	−		−			+?	1
Valine	+	+	+?	+	+	+	+	7?
Total	12	10	7	10	13	13	14	

*+, essential; +♂, essential to males; −, not essential; +?, probably essential.

Summary

Quantitative data on the amino acid contents of three susceptible varieties (Perfection, Daisy, and Lincoln) and three resistant varieties (Laurier, Champion of England, and Melting Sugar) of cultivated peas (*Pisum sativum* L.) were obtained by paper chromatography. The varieties susceptible to the pea aphid, *Acyrthosiphon pisum* (Harr.), generally contained a higher concentration of free and total amino acids than the resistant ones at the stages of growth corresponding with the period of pea aphid infestation in the field. It is suggested that the lower concentration of amino acids in the resistant varieties reduces the rate of aphid growth and reproduction and therefore contributes to the resistance of these varieties.

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Effects of Moisture during Starvation of Larvae of the Pale Western Cutworm, *Agrotis orthogonia* Morr. (Lepidoptera: Phalaenidae)[1]

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Larvae of the pale western cutworm, *Agrotis orthogonia* Morr., feed almost entirely below ground, attacking their food plants just below the soil surface. Larval movement and feeding usually occur at the interface between dry and moist soil. They can absorb moisture from the soil and also from the plants on which they are feeding.

In a previous investigation, Jacobson (1952) found that mortality from starvation varied directly with temperature and inversely with the size of larvae when the relative humidity was kept near 100 per cent. This paper is a report on the role of moisture during starvation.

Methods

After hatching, the larvae, in groups of 10 in 60-by-15-mm. petri dishes, were fed daily on sprouts of Marquis wheat. After moulting to the second or fourth instar they were allowed to feed for another two days before they were assigned to various humidities for starvation.

During starvation the larvae were confined singly in cages made from two-inch lengths of glass tubing, half an inch in diameter, with plastic or bronze screen cemented to one end. For fourth-instar larvae, 32-mesh screen was used but for the second-instar larvae 100-mesh was required. The cages, grouped as replicates, were placed in desiccators at the required relative humidity and supported by rigid screen wire holders.

Three levels of relative humidity were obtained in the following manner: low humidity with calcium chloride crystals in the bottom of the desiccator, medium humidity with 30 per cent concentrated sulphuric acid by volume, and high humidity with distilled water. Frequent measurements during the experiments showed the relative humidities to be about 5, 53, and 97 per cent, respectively.

At each of the three levels of relative humidity 5 replicates of 10 larvae were used in starving the fourth-instar larvae and 4 replicates of 15 larvae in starving the second-instar larvae. Each larva was weighed before and at regular intervals during starvation. Daily observations were made for moulting and mortality, and the dead larvae were weighed as soon after death as possible.

The loss of water during starvation at the three relative humidities was obtained by weighing groups of 10 larvae before and after various periods of starvation, followed by drying to constant weight in a vacuum at 100°C.

The percentage water content of feeding larvae, prepupae, and pupae was obtained by weighing groups of not less than five and drying to constant weight.

All experiments were conducted at a temperature of 25°C., obtained in a "walk-in" constant temperature room where the variation was less than one degree.

Statistical analyses were conducted by methods outlined by Cox (1954).

Results

Mortality during Starvation

Mortality during starvation was affected by the amount of atmospheric moisture (Fig. 1, Table I). With both second- and fourth-instar larvae the rate

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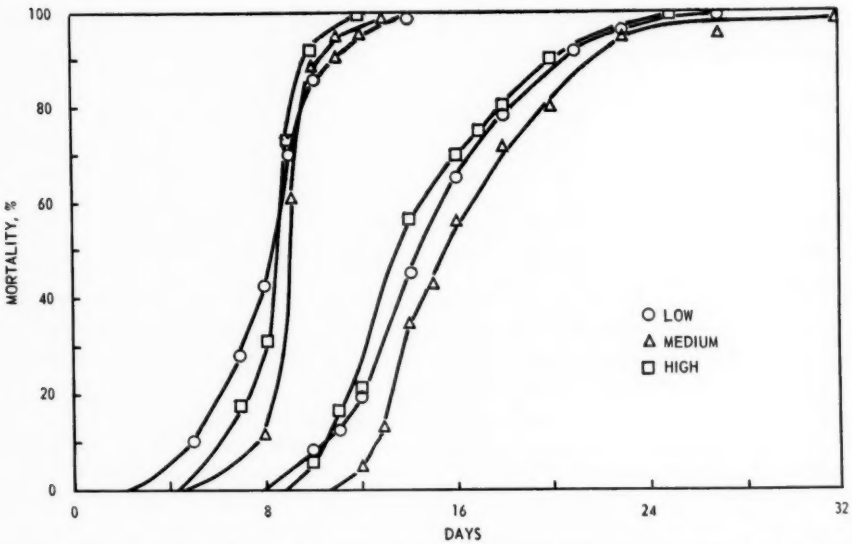

Fig. 1. Mortalities of second- and fourth-instar (left and right respectively) larvae of the pale western cutworm when starved at various humidities (low, 5%; medium, 53%; high, 97%) at 25°C.

of mortality was lowest at the medium humidity (Table I). The average numbers of days to death in Table I were calculated from the data shown in Fig. 1.

The data also indicate that fourth-instar larvae are more resistant to desiccation than second-instar larvae. In both instances excessive moisture was equally as harmful as severe desiccation.

Weight Loss and Moisture Content

At all humidities the loss in weight was very rapid during the first two days (Table II). Most of this loss in weight was presumably due to elimination of food products, since most of the faecal pellets were passed during this time. After this initial period, loss in weight continued at steadily decreasing rates. The amount of loss throughout the starvation period varied inversely with the relative humidity.

When single larvae were weighed it was noted that a very rapid loss in weight occurred immediately before death. During routine weighing of a group of larvae on the tenth day of starvation it was found that two larvae

TABLE I

Average numbers of days to death of second- and fourth- instar larvae of *A. orthogonia* Morr. when starved at three levels of relative humidity at 25°C.

Instar	Low R.H.	Medium R.H.	High R.H.
Second*	8.58	9.38	8.80
Fourth**	16.06	17.20	15.70

*Differences necessary for significance at 5% level, .32; at 1% level, .48.
**Difference necessary for significance at 5% level, 1.39.

TABLE II
Percentages of weight lost by fourth-instar larvae of *A. orthogonia* when starved at three levels of relative humidity at 25°C.

Relative humidity	Number of insects	Days				
		2	6	10	14	20
Low (5%)	50	17.6	33.9	46.8	53.0	57.3
Medium (53%)	50	12.9	22.0	30.9	40.6	46.8
High (97%)	50	9.7	15.0	21.2	28.0	32.6

at the high humidity had lost much more weight than the others in the replicate. They had lost 21 per cent of their initial weight between the sixth and tenth days compared with 7 per cent for the remainder of the larvae during the same interval. The two larvae died on the eleventh day and lost another 18 per cent of their original weight in the 24 hours preceding death. In total, these larvae had lost almost 40 per cent of their initial weight in 4 days.

Review of the available data on weight lost before and after death indicated a rapid acceleration of loss in weight immediately before death (Table III). The relationship of weight lost to the humidities at which the larvae were starved remained the same as indicated in Table II.

Both in the second- and in the fourth-instar larvae, the dry weight after a period of starvation was the same for all humidities (Table IV). The weight loss of fourth-instar larvae at the various humidities was almost identical with that shown in Table II. The percentage water content after starving varied directly with relative humidity. Analysis of variance showed that the differences in water content between the low and medium and between the medium and high humidity were significant at the 1% level.

When larvae are feeding they contain a high percentage of water, ranging from about 83 to 93 per cent of the total weight. In the quiescent stages of prepupae and pupae the water content is less. In the prepupae and pupae, daily weighings showed that weight was lost slowly and constantly until the moth emerged.

TABLE III
Percentages of weight lost by starved fourth-instar larvae of *A. orthogonia*, before and after death, at three levels of relative humidity at 25°C.

Relative humidity	Number of insects	Before death		After death
		2 days	1 day	1 day
Low (5%)	18	53	62	68
Medium (53%)	13	43	50	63
High (97%)	20	25	31	41

TABLE IV

Weights and water contents of second- and fourth- instar larvae of *A. orthogonia* when starved at three levels of relative humidity for various periods (10 insects for each test)

Instar	Days of starvation	Relative humidity*	Mean live wt. before starving mg.	Mean live wt. after starving mg.	Percentage wt. lost during starving	Mean dry wt. after starving mg.	Percentage water content after starving
Second.....	3	Low	31.2	19.5	37.5	2.7	86.1
		Medium	31.9	20.3	36.3	2.1	89.7
		High	27.7	22.2	17.5	2.1	90.5
	6	Low	33.1	13.8	58.3	2.4	83.6
		Medium	32.7	20.0	38.8	2.3	88.5
		High	29.8	21.5	27.8	2.3	89.4
Fourth.....	5	Low	51.4	38.6	24.9	5.4	86.0
		Medium	52.0	44.2	16.0	5.2	88.2
		High	54.6	51.4	5.9	5.6	89.1
	10	Low	52.0	31.8	38.8	4.0	87.4
		Medium	53.2	37.6	29.9	4.2	88.8
		High	57.4	48.0	16.4	4.8	90.0

*Low, 5%; medium, 53%; high, 97%.

Discussion

Insects manifest considerable variation in their ability to survive desiccation. Ludwig and Landsman (1937) found that when larvae of *Popillia japonica* Newman were starved at various humidities the length of life varied with humidity, ranging from four days at low humidity (about 5 per cent) to over a month at a humidity of 96 per cent. When nymphs of a grasshopper, *Chortophaga viridifasciata* De Geer, were starved, Ludwig (1937) found that death occurred in five or six days, regardless of humidity. In reviewing these data, Bellucci (1939) concluded that the difference in mortality between grasshopper nymphs and Japanese beetle larvae was due to natural differences in development and feeding habits. Grasshopper nymphs require constant feeding and, as there is little storage of fats, death from starvation occurs in a short time. The Japanese beetle must store enough food to sustain it during the pupal period and it draws upon stored fat when starved. Bellucci (1939) found that there were no differences in respiratory metabolism between fasting larvae at different humidities or of different water content.

The difference in time required for 50 per cent mortality of second- and fourth-instar larvae of the pale western cutworm after starving at the low humidity may be related to the ratio of surface area to weight. Ludwig (1945) states that a larger animal, because of its slower rate of water loss, is better able to survive exposures to low atmospheric humidities than a smaller one. One explanation for the greater resistance of the fourth-instar larvae to desiccation may be that they possess a greater reserve of fat that could sustain them for a longer period.

During starvation loss of dry matter and loss of water occur simultaneously. At high humidities loss in weight was probably due to the breakdown of tissues whereas at the medium and low humidities the proportionately greater loss was due to the increasing effect of desiccation. Unlike many other insects, such as starved larvae of the Japanese beetle as reported by Bellucci (1939), in which the difference in survival between various humidities is the effect of desiccation,

Table IV indicates that mortality of the pale western cutworm is largely a matter of starvation and the accelerated mortality at low humidity is the additional effect of desiccation.

These experiments show that a very moist environment is equally as harmful to larvae of the pale western cutworm as extreme desiccation. Ludwig (1945) mentioned that saturated air is usually unfavourable because it favours the growth of parasitic fungi. This did not occur in these experiments but may be a factor in the field.

Seamans (1935) based a method of forecasting outbreaks of the pale western cutworm on the fact that rainfall brings larvae to the surface where they are exposed to parasites and predators whereas Cook (1930) attached equal importance to the role of fungous and bacterial diseases. When there is a gradient from dry to moist from the surface downward, the larvae move to that level in the soil that provides the preferred environmental conditions. The experiments described herein have shown that moist conditions can be harmful. In years when rains are plentiful and the soil is soaked for long periods, mortality apart from parasites, predators, and disease may be greater in spite of an adequate supply of food. Hence, the optimum conditions for development and survival may possibly occur when the soil is dry and the larvae must consume large quantities of food to maintain their water content. In this way essential nutrients would be consumed and retained for the pupal period.

Summary

When larvae of the pale western cutworm, *Agrotis orthogonia* Morr., were starved at 25°C. at three levels of relative humidity, low (5 per cent), medium (53 per cent), and high (97 per cent), the rate of mortality varied. The rate was lowest at the medium humidity, and was almost the same at the low as at the high humidity. The fourth-instar larvae were more resistant to desiccation than the second-instar larvae.

Most of the weight lost was in water; the loss in dry matter was the same at all humidities, whereas water loss varied inversely with the relative humidity.

Acknowledgments

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Mochlonyx velutinus (Ruthe) (Diptera: Culicidae), an Occasional Predator of Mosquito Larvae[1]

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Though various species of the genus *Mochlonyx* Loew are known to be predacious on mosquitoes, there are only a few records of *M. velutinus* (Ruthe) (= *M. culiciformis* (DeG.)) in this role. According to Meinert (1886) the larvae of this species feed on the crustaceans *Cypris* and *Daphnia* in Denmark. At Churchill, Manitoba, Hocking *et al.* (1950) considered it the most abundant chaoborine in both open forest and tundra pools but did not specify its feeding habits. Twinn (1926) and Curtis (1953), however, referred to it definitely as a predator of mosquito larvae.

The habits of the predator were evaluated at the Belleville laboratory as part of a program of studies on the natural enemies of mosquitoes. This paper brings together results obtained from 1951 to 1954 with emphasis on the immature stages, population densities, life-cycle, habits, and predator-prey relationships.

The study was centred near Chatterton (Marsh Hill), Ontario, in a small section of a swamp (Fig. 1) in Lot 26, Concession VI, Sidney Township, Hastings County. In early spring, numerous pools form in this swamp from melting snow and ice. Some of these merge into larger pools in late March; other, smaller ones become separated and dry up as the season advances. Some permanent, deeper pools are less shaded than others by forest cover and contain a more varied insect fauna. All the pools are well-populated with

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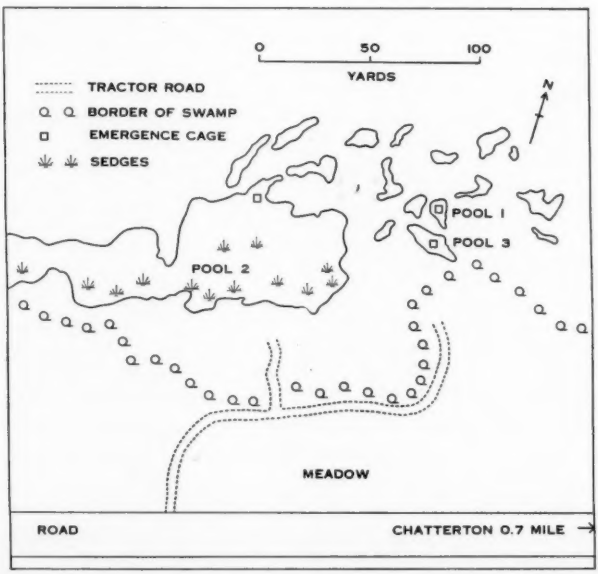


Fig. 1. Mosquito study pools, Lot 26 in Concession VI, Sidney Township, Hastings County, Ontario.

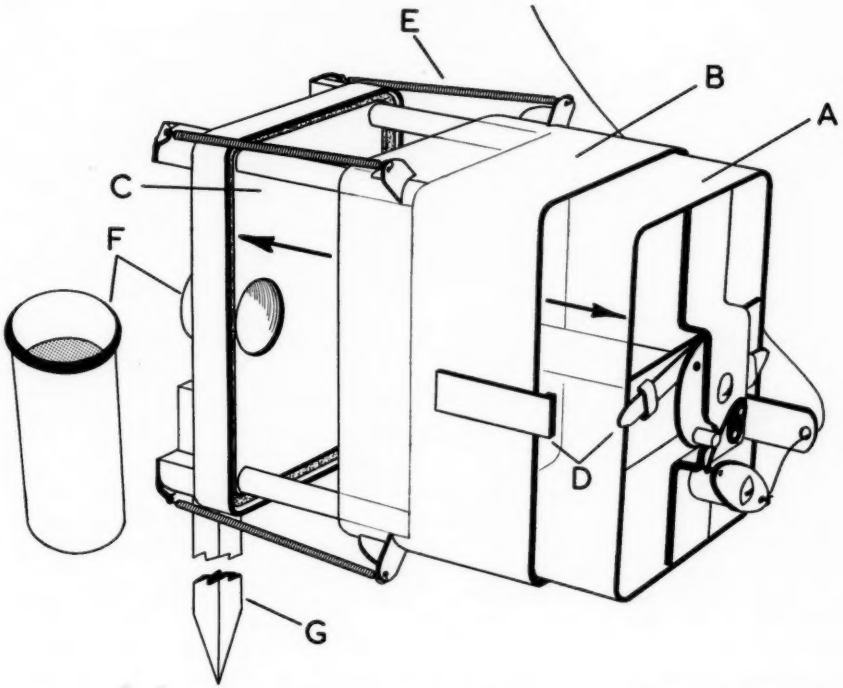


Fig. 2. Water displacement trap used in sampling larvae of *M. velutinus*, showing trap compartment open.

larvae of two important pest species of mosquitoes, *Aedes stimulans* (Walk.) and *A. trichurus* (Dyar), the former being considered the most abundant. Larvae of *A. implicatus* Vockeroth and *A. excrucians* (Walk.) are found much less frequently.

Equipment and Methods

Larvae of the predator were sampled with a new type of water-displacement trap made of plastic (Lucite; Johnston Industrial Plastics Limited, Toronto, Ontario). This trap (Fig. 2) consists of two boxlike sections, one telescoped within the other. The inner section (A) holds a tripping mechanism at one end, and is open toward the other end on four sides except for the corner supports. The outer section (B) slides over the inner one and has four solid sides, which, when drawn by springs (E) to the end of the inner section, forms the trap compartment (C). When the trap is set, the outer section is withdrawn under spring tension and held by catches (D) connected to the tripping mechanism, leaving the trap compartment open.

The tripping mechanism (Fig. 3) is attached to two cross struts. On the lower strut (A) is a rotating disk with two movable rods (B). By slightly rotating the disk anti-clockwise, the end of each rod is guided through a sleeve to a catch (D) on the sliding section of the trap. The rods are released indirectly by a steel, leaf spring (C) mounted on the upper strut (E) and held under tension by a small metal pin (F) on a movable arm (G). Attached to the opposite end of the arm are two yards of light nylon thread.

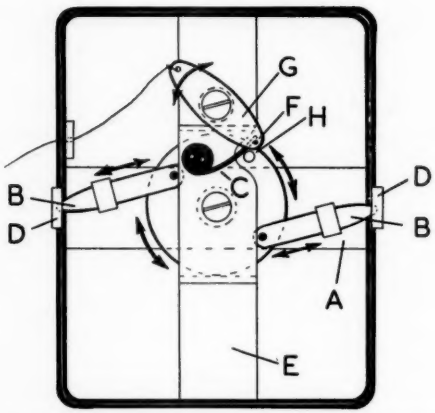


Fig. 3. Plan of tripping mechanism of water displacement trap.

After the trap has been set, a gentle pull of the tripping thread releases the spring to strike a plastic knob (H) on the disk below. This in turn rotates the disk, releases both rods, and quickly closes the trap compartment. A sample of approximately one litre of water is taken.

After the sample has been taken, the catch is strained into a removable plastic vial (Fig. 2, F) open at both ends. The trap may be placed on the bottom of a pool or may be attached to a wooden stake (Fig. 2, G) and set at lesser depths.

Samples were taken each morning at ten-minute intervals for two hours from representative populations of the predator. The pools were sampled from May 1 to 5, 1951.

Cages were used in the pools to determine the period of emergence and the relative abundance of the predator and the pest mosquitoes. A special plastic screen cage covering an inside area of 0.5 square metres, and a walk-in cage of four square metres were placed in two temporary pools; a second plastic cage was set in a permanent pool. Adults were removed from the cages every two days.

The square, plastic screen cage is of wooden-frame construction (Fig. 4) and consists mainly of a base (A), a removable top section (B), and a separator (C) for each section. The corners of the base extend below the level of the screen as legs, and project somewhat above it to hold the top section in place. Near the top of the base in front is a narrow opening through which a sliding separator is inserted and moves along grooves on the inside of the frame. A similar separator fits grooves at the base of the top section. The separators are made of one-eighth-inch, tempered, pressed fibreboard. The base, except at the corners, is 20.5 cm. high, and the top section 50 cm.

In setting up the cage, the legs of the base are forced into the bottom of the pool and heavy sods are placed underwater around the base to prevent the escape of any culicid larvae. The top section is then applied and fastened tightly to the base on three sides by flat, brass hooks, the two separator openings being closed with wooden plugs (D).

To remove the catch from the trap, the plugs are withdrawn and the separators slipped into place. The top section is then unhooked and replaced by an empty one without releasing any adults that have emerged in the lower section.

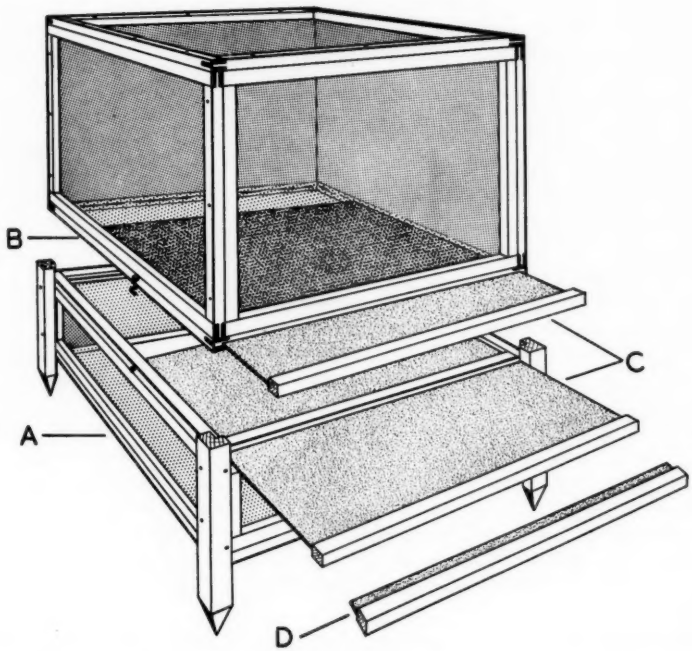


Fig. 4. Plastic screen emergence cage, with removable top, showing separators partly withdrawn.

Data on the mating behaviour of the predator were obtained in the swamp from May 30 until June 18, 1951, mainly during late afternoon and evening. One hundred and twenty-five adults of each sex were placed in each of four wooden cages (18 x 12 x 12 inches) covered with cheesecloth except for plastic fronts. During the observations, temperature and humidity were recorded one foot above the ground with a Taylor hygrometer at ten-minute intervals; light intensity was measured with a Weston meter.

In the laboratory, ovipositing females were put into Melrose rearing boxes held at 25°C. and 80-85 per cent relative humidity, and fed diluted honey. Eggs laid in the boxes were transferred in lots of 50 to moist blotting paper in 2-inch vials, kept at 25°C. for 12 days, and then placed in storage at 0°C. After six months, batches of eggs were removed from storage and were incubated in pond water at 19°C. Resulting larvae were isolated in 8-ounce glass jars and reared at 8°, 12°, and 18°C. The width of the head capsule and the length of the larvae at eclosion were measured from preserved specimens; measurements were made similarly of latter instars.

The feeding habits of the predator were studied by examining the contents of the digestive tract. During April, collections of larvae of all stages were preserved soon after capture in a formol-acetic-acid mixture. These were dissected later to identify the contents of the crop and fore-intestine.

Field data on the larval growth of the predator and of the culicines were obtained from collections made every five days. The head widths of all larvae were measured to compare growth in each species, these falling naturally within

four well-defined groups corresponding to the larval instars. The lengths of the larvae, i.e., from the front of the head to the tip of the siphon, were measured also for comparing growth of the predator and the *Aedes* larvae.

Descriptions of Immature Stages

Egg

The egg is creamy white, elongate-ovoid in shape, and slightly larger toward the anterior end. The chorion is sculptured into narrow hexagonal areas. When newly laid it is 0.61 mm. in length and 0.13 mm. in greatest width. Before hatching, the eggs attain a length up to 0.73 mm. and a width up to 0.17 mm.

First-instar Larva

The first-instar larva is of the eruciform type with a well-sclerotized head, three thoracic and nine abdominal segments (Figs. 5 and 6). This stage is distinguished by the relatively narrow thorax, only slightly wider than the head, long body setae, a cone-shaped respiratory tube one and one-half times longer than broad at the base, and a ventral brush with extremely long hairs. Each antenna bears four terminal bristles, one of which is lanceolate and very short (Fig. 8). The mandible (Fig. 9) has a strong apical tooth bearing a pair of minute inner teeth, as well as a short basal tooth and three or four fringed setae. No mandibular fan is present, but in its position is a pair of small setae. There is a single pair of median frontal (labral) setae, which are widely separated at the base (Fig. 8). At eclosion, the larva is 1.6 mm. long and the head capsule and thorax 0.28 m. and 0.3 mm. wide respectively.

Second-instar Larva

The second-instar larva is 3.4 mm. long and has a maximum width of 0.7 mm. (Fig. 7); the width of the head capsule is 0.52 mm. In contrast to the first-instar larva, the thorax is considerably wider than either the head or the abdomen, the body hairs are short, and the respiratory tube is two and one-half times longer than broad at the base. The antenna is terminated by four slightly curving bristles, one of which is pointed and two-thirds as long as the others. In this stage the mandible has five well-defined teeth, four fringed setae, a short basal spine, and a mandibular fan of five simple hairs.

Third-instar

The third-instar larva resembles the second-instar larva in many respects. The thorax is much wider than the head, and the respiratory tube relatively narrower, its length being almost four times the width of its base. The larva is 5.2 mm. long and has a maximum width of 1.0 mm.; the width of the head capsule is 0.87 mm. The mandible is armed with six or seven strong teeth and the mandibular fan consists of five or six simple hairs.

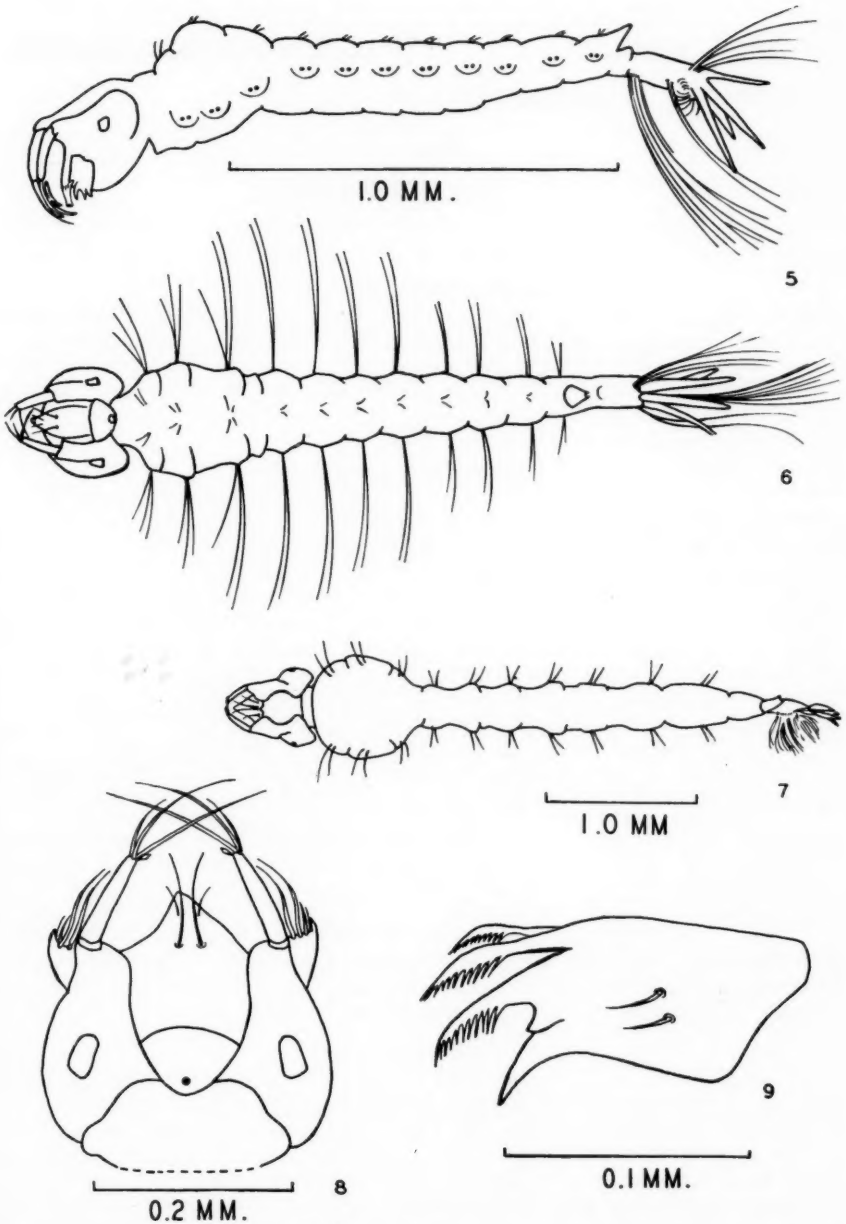
Fourth-instar Larva

The mature larva and pupa were described first by Meinert (1886), but in greater detail by Cook (1956). The former stage is readily separated from all others by the width of the head capsule and by the presence of two adjacent pairs of median frontal setae.

Specimens were from 5.9 mm. to 8.7 mm. long, the head capsule varying from 1.25 mm. to 1.38 mm. in width. The mandible has seven or eight teeth and a mandibular fan of six or seven hairs.

Population Density

The predators were numerous among the large populations of culicine larvae in both temporary and permanent pools. Because of their relative immobility they were more readily sampled than the culicines, which tended to



Figs. 5-9. *M. velutinus*. 5, First-stage larva, lateral aspect. 6, First-stage larva, dorsal aspect. 7, Second-stage larva, dorsal aspect. 8, Head, first-stage larva, dorsal aspect (partly diagrammatic). 9, Left mandible, first-stage larva.

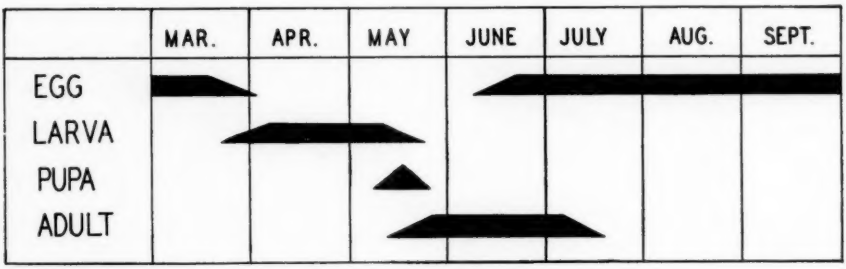


Fig. 10. Life-cycle of *M. velutinus*.

disperse when the sampler was placed in the water. The larvae of the predator were much less disturbed by this device than were the culicines and were often observed to enter the trap after it had been set. Culicine larvae were frequently taken in the trap, however, but not in proportion to their numbers in the pool.

The predator was more abundant in the temporary than in the permanent pools. Population densities in three pools were:

Pool	No. of Larvae	Mean No. per Litre
Temporary (No. 1)	23 (13 samples)	1.8
Permanent (No. 2)	14 (20 samples)	0.7
Temporary (No. 3)	50 (20 samples)	2.5

In the large, permanent pool, the larvae were uniformly present in the shallow water but less numerous in deeper water two to three metres from the margin. According to Baldwin, James, and Welch (1955) the larger predators, especially dytiscid beetles, prey upon the culicines in this pool. Probably many larvae of *M. velutinus* are destroyed also. On the other hand, the high concentration of the predator larvae in a temporary pool (No. 3), as in many other shallow pools, resulted from a drop in the water level due to evaporation.

Differences in the population densities in each pool were reflected later in the numbers of adults per litre that emerged in the cages, as follows:

Pool	*Aedes* spp.	*M. velutinus*
Temporary (No. 1)	33.3	1.5
Permanent (No. 2)	1.97	0.5
Temporary (No. 3)	-	2.2

These values show that the numbers of adults per unit of volume were less than those of larvae taken in the water displacement trap. The discrepancies are attributed to other predators observed in the cages and to the presence of small oil slicks, which may have delayed emergence.

Life-Cycle

M. culiciformis has one generation a year and overwinters in the egg stage (Fig. 10). The eggs are laid in early June in moist soil and under fallen leaves in and near the breeding pools. On June 5, many of the ovaries of some 25 females contained mature eggs, but most of them were in various immature stages of development. The eggs develop somewhat during the summer and then enter diapause for about eight months. Hatching occurs with the flooding of the pools during the latter part of March, shortly after the first appearance of the culicine larvae.

In the laboratory, females caged on June 5 began to oviposit on June 18, 13 days after mating. The eggs were laid singly and at random on moist

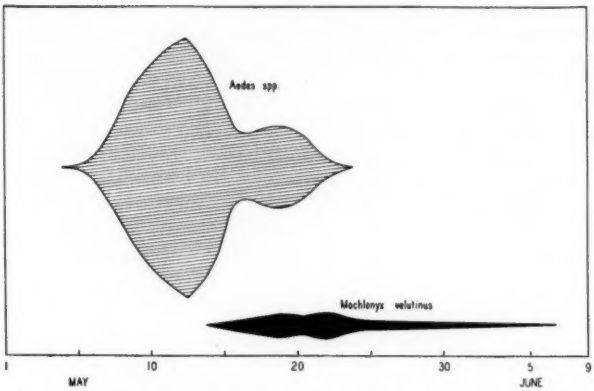


Fig. 11. Succession and relative abundance of adults of *M. velutinus* and *Aedes* spp. in pool No. 1, Chatterton, Ontario, 1951.

dental cotton as well as on the bottom of the Melrose box. Oviposition was not observed. From June 18 to July 6, 671 eggs were obtained from 84 females. Batches of eggs were incubated in tap water at 2.2°, 4.5°, and 19°C. after storage at O°C. for six months. At 19°C. eclosion occurred in 30 minutes. Many eggs remained viable for 18 months after storage at O°C.

Although populations of the predator require about six weeks to complete larval growth, further flooding may extend beyond two months the period in which the larvae are found.

In the laboratory, one lot of four larvae, reared in pond water at 8°C. and fed small crustaceans, attained the fourth instar in 29 days. Two lived 18 more days without pupating. A second lot of five larvae fed similar prey but reared in the dark at 12°C. reached the fourth instar in 32 days. These larvae grew most rapidly during the first two stadia (12 days) and though well supplied with prey none pupated, three living for 22 more days. The third lot, consisting of seven larvae, were reared at 18°C. and required only 14 days to reach the fourth instar. Some of these pupated 13 days later.

Field-collected mature larvae taken on May 5 and held at 21°C. became pupae in 24 hours. At this temperature the pupal period lasted four days.

Adults begin to emerge from the pools during early May, emergence continuing for about three weeks. In 1951, they did not appear in the cage in Pool 1 until 81 per cent of the culicines had emerged (Fig. 11).

Habits

Adult Habitat

The adults were not found outside the swamp, but both sexes were numerous among the vegetation surrounding the breeding pools. From 10 to 25 adults per square metre were readily collected with an aspirator from foliage of the plant cover. They generally preferred moderate shade to deep shade or bright sunlight. On dull days they were observed flying a few inches above the foliage and also resting on the upper leaf surfaces of cinnamon fern, *Osmunda cinnamomea* L.; poison ivy, *Rhus toxicodendron* L.; and dwarf raspberry, *Rubus triflorus* Richardson. On hot, sunny days they preferred the under-surface of the leaves but quickly flew out if the vegetation was disturbed.

TABLE I
Light-intensities and Temperatures at Which *M. velutinus* Mated in Field Cages

Date	Period of observation (Standard Time)	No. mated	Light intensity, ft-c.		Mean temp., °C.
			At mating	Range during period	
May 30	18.25–18.45	2	37	60 to 20	22.0
	19.30–19.50	0	–	2.8 to 0.4	16.1
May 31	18.25–19.45	18	26 to 10	26 to 1.6	22.0
June 1	14.45–15.15	0	–	820 to 220	23.8 (23.0–24.4)
June 5	18.20–18.50	5	25	25 to 19	18.0
June 8	14.30–16.30	0	–	1200 to 100	22.6 (20.0–24.4)
	17.00–19.00	6	60 to 38	300 to 28	18.2
June 10	5.30– 7.50	0	–	45 to 220	15.8 (11.0–17.8)
June 15	17.20–18.50	5	90 to 35	100 to 35	18.5
June 18	17.10–19.30	3	60 to 18	220 to 10	22.2

Mating

Caged males were in sustained flight (swarmed) at temperatures from 18° to 24.4°C. and at light intensities from 37 to 90 foot candles. Below and above this light level, swarming decreased. Only a few individuals were in flight at 19°C. when the light was reduced to 3.5 foot candles or at 17.7°C. when it had increased to 220.

Mating occurred in the evening (Table I). Though both sexes were exposed to light of approximately 1,200 to 0.4 foot candles, they mated only when its intensity fell within 90 to 10 foot candles. During mating the temperature varied from 16.7° to 23.3°C. and the relative humidity from 49 to 87 per cent.

Mating began during flight. Females that left the sides of the cage were quickly seized by males, the pairs dropping almost at once to the side or floor of the cage. A pair remained usually end to end, the female upright and the male either upright or lying on its back or side. Copulation lasted from seven to 45 minutes.

Larva

The larva is well adapted as an aquatic predator. Pairs of air sacs in the thorax and in the seventh abdominal segment store oxygen and also serve as hydrostatic organs that enable the larva to be suspended horizontally in the pool at various depths. As a rule the larvae remain more or less immobile in deeper water, where they capture small organisms that come within reach of their prehensile antennae. The prey are taken into the expansive foregut and digested, the skeletal remains being ejected by the eversible crop.

The larvae occasionally change their positions by a sudden twist of the body. Although they resemble culicine larvae in having a respiratory tube, they were not observed to use this organ at the surface film, at least during daylight hours.

Food

Dissection of larvae taken from temporary pools in 1953 showed that they had fed extensively on crustaceans, as follows:

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TABLE II
Stages and Lengths in Millimetres of Culicid Larvae from Temporary Pools, Chatterton, Ont., 1953.

Stage	*M. velutinus*		*A. stimulans*		*A. trichurus*	
	Mean	Range	Mean	Range	Mean	Range
I	2.1	1.6–3.0	3.1	2.2– 3.7	3.5	2.8– 5.2
II	3.4	3.3–4.7	5.2	5.0– 6.1	5.9	3.8– 6.3
III	5.6	4.5–6.2	7.8	5.3– 9.4	8.2	5.4– 8.5
IV	6.3	5.7–8.7	9.3	8.5–10.2	10.0	9.2–11.2

Date	April 9	April 13	April 15	April 18	April 24	April 27
Number dissected	15	8	23	10	7	13
Crustaceans	56	21	48	20	6	8
Other prey	3	2	1	1	1	2

The prey consisted largely of ostracods, copepods, and cladocerans, the ingestion of these animals causing the gut in living larvae to appear typically yellow. In general, the earlier stages contained more immature crustaceans and smaller organisms such as rotifers and *Volvox* spp. than the later. Fewer and larger prey were found in the third- and fourth-stage larvae. No remains of mosquito larvae, however, were identified. This agrees with results obtained in 1953 by Baldwin, James, and Welch (1955) by means of a radio-active tracer.

M. velutinus, however, preyed on mosquito larvae in 1953 near Bancroft, Ontario, and in the Chatterton pools in 1954. The Bancroft collections consisted of third-instar larvae taken from deep pools in the valley of the York River. Approximately 14 per cent contained remains of first-instar larvae of *Aedes* spp. In many of the predators the dark remains of the mosquito larvae in the crop were observed through the transparent body wall. At Chatterton, predation on mosquitoes occurred after an additional hatch of eggs was favoured by flooding of the pools in mid April. Dissection of third-instar larvae of the predator revealed the remains of head capsules of recently hatched culicine larvae, some of which were clearly those of *Aedes trichurus*. Before the pools had flooded, the predators had fed exclusively on *Cyclops* spp. and other crustaceans. Predation occurred also in dense populations of culicine larvae in test cages set among sedges in a permanent pool. In a collection of 90 predators made on April 30, 16 contained culicine remains.

Relative Size of the Predator

The lack of predation by *M. velutinus* on culicines in a normal season is believed due to its relatively smaller size. On the assumption that the size of a culicid larva is proportional to its length, comparisons of mean lengths of 470 larvae of the three species taken from mixed populations showed that the predator is smaller than the culicine larvae at all stages (Table II); similarly, the larvae of *A. stimulans* are smaller than those of *A. trichurus*. Nevertheless, though the eggs of the three species normally hatch about the same time, the lengths of the larvae range widely during development, so that the largest of *M. velutinus* are subequal in size to some of the culicines. Possibly some predation may occur also under these conditions.

Depending on its relative size, *M. velutinus* is an occasional predator of culicine larvae. At Whitehorse, Yukon Territory, Curtis (1953) observed that overwintered larvae of this species, presumably of a late instar, quickly eliminated newly hatched culicines. Similarly, Jackson (1953) found in the laboratory that the number of larvae of *Aedes aegypti* (L.) eaten by the culicid *Lutzia tigripes* G. & C. depended on the size of the prey, the smaller prey being eaten in larger numbers.

As *M. velutinus* does not attack culicine larvae at Chatterton during a normal spring, however, it is concluded that this species is not a significant predator of biting mosquitoes in that area.

Summary

The life-cycle, population density, and habits of *Mochlonyx velutinus* (Ruthe) were examined at Chatterton, Ontario, where this species is closely associated with *Aedes stimulans* (Walk.) and *A. trichurus* (Dyar). During larval growth, the appendages of the head are modified, the thorax becomes relatively wider, the respiratory tube narrower, and the body setae shorter.

The number of larvae taken per litre with a new type of sampling apparatus approximated the number of adults that emerged per litre in trap cages. More of *M. velutinus* emerged from shallow pools in which the larvae had become concentrated than from permanent ones in which larger predators were more numerous.

During the day, adults are found in the vegetation surrounding the breeding pools; mating occurs in late evening. The larvae are well adapted to capture zooplankton. They do not usually attack culicines, but may destroy small larvae of late broods. This species, however, is not a significant predator of biting mosquitoes during a normal spring.

Acknowledgments

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A Simply Constructed Vessel for Freeze-Drying[1]

By W. G. Friend[2] and T. M. B. Payne[3]

Freeze-drying has become widely accepted as a means of concentrating and preserving substances without destroying biological activity, and is becoming increasingly important in various entomological and bacteriological studies. The methods and types of apparatus used have been recently reviewed by Harris (1954). The apparatus described in this paper was developed at the Entomology Laboratory, Ottawa, for drying small quantities of insect tissue. It is sturdy and may be easily and cheaply constructed from readily available laboratory supplies with common machine-shop tools.

The vessel consists of two stainless steel beakers fitted one inside the other and soldered to a circular brass casting (Figs. 1 and 2). The inner side of the casting is tapered so that the coolant medium may be easily poured from the vessel and also that "boiling over" is less likely when dry ice is added as the coolant. Thirteen vacuum stopcocks are mounted spirally in the outer beaker. This arrangement minimizes weakening of the beaker and permits convenient arrangement of the glass flasks containing the material to be dried. The stopcocks must be very tight to maintain a high vacuum, and consequently the wrench illustrated in Fig. 2 is useful to manipulate them.

Thick walled, 250-ml. centrifuge bottles fitted with rubber stoppers containing short lengths of glass tubing have been used as drying flasks. These

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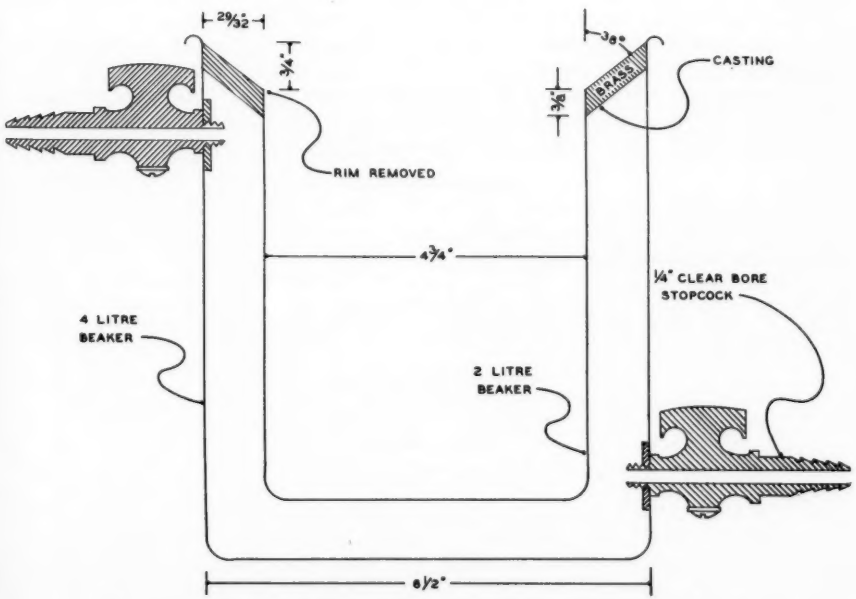

Fig. 1. Cross-section of freeze-drying vessel giving construction details.

Fig. 2. Side view of vessel showing attachment of centrifuge bottle and wrench.

flasks are connected to the stopcocks with short pieces of heavy-walled rubber tubing (Fig.2). Ethylene glycol monomethyl ether is a satisfactory medium for the coolant, dry ice.

In operation, the vessel is connected, through a series of vapour traps, to a high-vacuum pump. The inner beaker is three-quarters filled with the coolant liquid, to which pieces of dry ice are added. The material to be dried is shell-frozen to the sides of the centrifuge bottles by swirling them in the coolant liquid. The bottles are then connected to the vessel (Fig. 2).

This apparatus is capable of drying 25 ml. of a 10 per cent salt solution per stopcock in four hours. Ice forming in the interior of the vessel tends to block the stopcocks and prevents the drying of more than 25 ml. at any one stopcock. After use, the coolant is removed and the interior of the vessel is flushed with warm water and drained to remove the ice.

Acknowledgments

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Notes on Rearing a Pupal Endoparasite, *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae), on Unnatural Food[1]

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Eggs of the ichneumonid endoparasite *Pimpla turionellae* (L.) were observed by Bronskill to develop and hatch in physiological saline solution. This raised the question whether the larvae could be reared on unnatural food. Early attempts (Simmonds, 1944) to rear ichneumonid ectoparasites on nutritive gelatin slopes and raw beef were unsuccessful. Heuristic attempts at Belleville have succeeded in raising a small number on such a food.

The larvae were fed a slurry of pork liver and 0.8 per cent aqueous solution of sodium chloride. Equal parts by weight of liver and saline solution were homogenized in a metal Waring Blendor and autoclaved at 15 pounds pressure for 15 minutes. When cooled, the coagulated homogenate was transformed into a thick watery mixture without breaking asepsis. About 2-ml. aliquots of this were pipetted into sterile test tubes, 12 mm. in diameter, and plugged with non-absorbent cotton. Embryos of the parasite, dissected from parasitized pupae of *Galleria mellonella* (L.), were dipped momentarily into a mercuric chloride solution (White, 1937, p. 423) and rinsed four times in sterile saline solution with a camel's-hair brush. They were then transferred to the test tubes for rearing singly.

All rearing was done in the dark at 23°C. with a relative humidity of about 72 per cent. The embryos hatched within a few hours and began to feed. Survival was greatest when the larvae remained on the surface of the food with only a film of liquid moistening them. When each larva matured it was transferred to a small gelatin capsule lined with blotting paper. Several minute holes were pierced through the ends of each capsule for ventilation. The parasite completed metamorphosis within the capsule.

Of the 152 embryos handled in this manner, 147 hatched. Ten (7 per cent) of these became adults. For comparison, 20 adults (49 per cent) of *P. turionellae* were obtained from 41 parasitized pupae of *G. mellonella*. Mortality on the medium occurred during all stages of larval and prepupal development but normal pupae always formed well-developed adults. One of the females produced viable offspring and lived more than three months.

This work shows that the endoparasite *P. turionellae* can be reared on an unnatural food. The physical and chemical properties of the food medium are not entirely satisfactory. It was found advantageous to cook the liver to overcome the viscosity of its juices. Moreover, a wet, particulate medium was the most satisfactory food tested. Work is being continued to determine the nature of other factors necessary for optimum growth and development.

Acknowledgment

We are indebted to Mr. F. K. Seemungal for technical assistance.

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A Unique Association of Two Species of Lepidoptera[1]

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On August 15, 1955, during studies on the ecology of caterpillars on cabbage near Carp, Ontario, two species of Lepidoptera were observed in unique association. A final-instar larva of the diamondback moth, *Plutella maculipennis* (Curt.), bore on its third abdominal tergite an egg of the imported cabbageworm, *Pieris rapae* (L.) (Fig. 1). The larva appeared to be unaffected by the presence of the egg and when brought into the laboratory spun its cocoon within 24 hours. The egg remained attached to the larval skin, becoming enclosed within the cocoon, and hatched during the prepupal period. The resultant larva tried to free itself from the cocoon for several hours and eventually died.

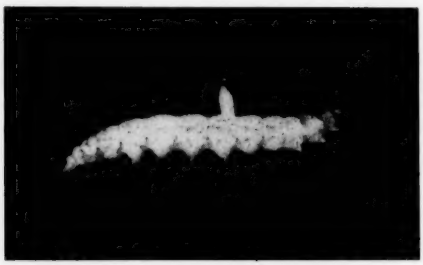

Fig. 1. Fourth-instar larva of *P. maculipennis* with egg of *P. rapae*.

This phenomenon may be explained by the indiscriminate egg-laying habit of the cabbage butterfly. Frequently, the female lands on the edge of a leaf and curves her abdomen downward until the tip of her ovipositor touches the lower surface. At the moment of impact a single egg is glued to the leaf. As the final-instar larva of the diamondback moth feeds most commonly on the lower surface of the leaf (Harcourt, 1954), it is remarkable that this phenomenon does not occur more often. This was the first evidence of its occurrence in examinations of more than 75,000 larvae of the diamondback moth,

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